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U. S. ARMY COMPONENT
SEATO MEDICAL RESEARCH LABORATORY
APO 146, SAN FRANCISCO, CALIFORNIA.

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QUARTERLY RESEARCH PROGRESS REPORT

1 January 1963 — 31 March 1963



SUBACTIVITY OF :

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This Compilation of Scientific Data represents some of the research accomplishments of the

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Major General Pung Phintuyothin, MC, of the SEATO Medical Research Project, is the Director General and Director of Thai Component

Colonel James L. Hansen, MC, is the Director of U.S. Component SEATO Medical Research Laboratory

Inquiries or comments concerning the work reported in this Quarterly Report should be forwarded to the Director, U.S. Component, SEATO Medical Research Laboratory.

28.10

US Component (Army)
SEATO Medical Research Laboratory
APO 146, San Francisco, California

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Department of Bacteriology & Immunology

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01, Communicable Diseases (FIC 410)

Subtask aj: Acute Gastroenteritis

Study 1. Bacteriologic Survey of Stools from
Patients with Acute Diarrhea (SEATO
MEDIC Study No. 60)

Investigators: Lt. Colonel Sidney Gaines, Dr. Chiraphun
Duangmani, Mrs. Tatsanee Occeno,
Miss Pranee Senadisai, SFC Wallace G. Fee.

Description:

To determine the types of enteric pathogens involved in
acute diarrheas in Thailand.

Progress:

Examination of stools from acute diarrhea patients in 9
Bangkok hospitals cooperating with this laboratory continued
during the period covered by this report. A total of 857 rectal
swabs from 601 patients (128 adults, 473 children) were submit-
ted to the laboratory. The results obtained show that from 111
(12.9%) of the swabs, 15 different Salmonella species were
isolated. The 111 positive specimens were obtained from 88
different patients, indicating that 14.6% of all the patients were
positive for Salmonella in one or more stool specimens submitted.
Fourteen of the 111 Salmonella were isolated from 16 adults,
while the remaining 97 were cultured from 72 children. Thus,
12.5% of the adult patients and 15.2% of the children were posi-
tive for these organisms. It is of interest to mention that in
two instances two different Salmonella species were isolated
from the same specimen, and that in two other cases a Salmonella
and a Shigella species were cultured from the same specimen.

The high incidence of diarrhea associated with Salmonella
montevideo, referred to in previous reports, decreased somewhat
during the Quarter in the Children's Hospital. Of the 240
patients from this hospital examined during this period, 26
(10.8%) were positive for this species. A total of 455
specimens were submitted from the Children's Hospital, and from
these, 37 S. montevideo were cultured. This represented 50.7% of
all the Salmonella (73 isolates) cultured from the specimens
submitted by this hospital. In the previous Quarter, 79.2% of
all the Salmonella cultured from patients in this hospital

Task 01, (Project No. 3A O 12501 A 806) Cont

were S. montevideo.

Seventeen Shigella isolates (4 from adults, 13 from children), representing 6 different Shigella species, were obtained from 17 patients (4 adults, 13 children). Thus, 2.8% of all the patients examined (3.1% of the adults, 2.7% of the children) were positive for these organisms.

Regarding the paracolons, 438 of these organisms were isolated from the 857 rectal swabs examined during January, February, and March. However, only 382 (79 from adults, 303 from children) of the 857 specimens were positive for paracolon species, and these were obtained from 311 patients (77 adults, 234 children). As many as 3 different species were obtained from a number of individuals, sometimes from the same sample. Approximately 60% of the swabs from adults (79 of 131) yielded paracolon bacilli, while approximately 42% of the swabs from children (303 of 726) were positive for these organisms. A total of 97 paracolons were cultured from the positive 77 adults, and 341 from the positive 234 children. Among the paracolon bacilli cultured were numerous isolates of Paracolobactrum coliforme, P. aerogenoides, and P. intermedium. Providencia strains, on the other hand, were found infrequently.

In addition to the above, many isolates of Proteus, Pseudomonas, and Bethesda-Ballerup bacilli were obtained from many of the specimens examined. In addition, occasional isolates of Arizona bacilli were made.

Summary and Conclusions:

This study is still under active investigation.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01, Communicable Diseases (FIC 410)

Subtask aj: Acute Gastroenteritis

Study 2. Occurrence of Enteropathogenic Escherichia coli in Thailand
(SEATO MEDIC Study No. 61)

Investigators: Lt Colonel Sidney Gaines, Miss Yupin Achavasmith,
Mrs. Malinee Thareesawasdi

Description:

To determine the kinds and distribution of enteropathogenic Escherichia coli isolated from acute diarrheas in Thailand.

Progress:

Serotyping of E.coli strains isolated from patients with acute diarrheas continued, employing 12 typing sera provided by Walter Reed Army Institute of Research. Prior to the present Quarterly Report, 957 E.coli had been typed. Of these, 115 (12.01%) were found to be enteropathogenic serotypes, and 155 (16.2%) were rough strains. During the Quarter beginning 1 January 1963, 311 additional cultures were examined. Seventy seven (24.8%) were shown to be enteropathogenic, while 115 (36.9%) were rough. To date, therefore, of 1268 E.coli tested, 192 (15.1%) enteropathogenic and 270 (21.3%) rough strains have been obtained. These cultures were isolated from individuals of various ages and from both sexes. The most common types found so far have been serotypes 025:B19:B23 and 0119:B14, representing together half (51.6%) of all the types isolated.

Summary and Conclusions:

This study is still in progress. It is apparent, however, that to date approximately 15% of the individuals with acute diarrhea have yielded enteropathogenic E.coli in their stools. The data are being analyzed and evaluated.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01, Communicable Diseases (FIC 410)

Subtask aj: Acute Gastroenteritis

Study 3. Diarrhea in U.S. Troops Newly Arrived in
Thailand
(SEATO MEDIC Study No. 62)

Investigators: Lt Colonel Sidney Gaines, Lt Colonel Robert
B. Giffin, Jr. (Letterman General Hospital)

Summary and Conclusions:

This study has been terminated. The data are being
analyzed and evaluated, and a publication on the findings will
be forthcoming.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01, Communicable Diseases (FIC 410)

Subtask aj: Acute Gastroenteritis

Study 4. Diarrhea in U.S. Personnel in Thailand.
(SEATO MEDIC Study No. 65)

Investigators: Lt. Colonel Sidney Gaines, Dr. Chiraphun
Duangmani

Description:

To determine the incidence and causes of diarrheas in U.S. personnel living in an area where diarrheas are endemic, and to investigate the development of diarrheas in U.S. personnel immediately after their arrival in Thailand.

Progress:

On 10 February 1963 an outbreak of acute diarrhea occurred in a U.S. military unit in the vicinity of Korat, Thailand. Eleven patients were initially involved and were admitted to the 31st Field Hospital in Korat. Of these, 8 were positive for Shigella sonnei 1. One of the patients was a cook and all had eaten all their meals in the company mess. However, the time of onset of symptoms in all the patients, including the cook, was much the same. Samples of all food served in the mess hall during the two days prior to the outbreak, along with a water sample from the unit mess, were cultured. In no instance were any enteric pathogens isolated. During the next 10 days, 4 additional members of the same military unit were cultured and found to have Sh. sonnei 1 in their stools. Antibiotic therapy consisted of either chloramphenicol or tetracycline, and all patients responded well within 24-48 hours. Since the 20th of February 1963, only 2 stool specimens have been received from patients with diarrhea through the 31st Field Hospital, and from neither of these could enteric pathogens be isolated.

Task 01 (Project No. 3A O 12501 A 806) Cont

From another diarrhea outbreak in Pitsanuloke in northern Thailand, specimens from 18 individuals associated with the JUSMAG unit there (7 U.S., including military personnel and their dependents, and 11 Thai) were received on 27 March 1963. These persons were suspected of having salmonellosis. By the time the specimens were obtained, however, most of the individuals had been on treatment and were asymptomatic. Despite this, Salmonella derby was isolated from a U.S. Army officer, and a Salmonella species was isolated for each of two of the Thai personnel. No information was available on the source of this outbreak.

Summary and Conclusions:

This study is a continual one, since outbreaks such as those described can be expected to occur from this time to time in U.S. personnel living in areas where diarrheas are endemic, and where a substantial portion of the local population harbor known enteric pathogens.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01, Communicable Diseases (FIC 410)

Subtask: Enteric Infections

Study 1. Bacteriologic Survey of Stools from Normal
Individuals
(SEATO MEDIC Study No. 63)

Investigators: Lt. Colonel Sidney Gaines, Dr. Chiraphun
Duangmani

Description:

To provide information on the types of enteric pathogens found in the normal Thai population, and to provide a basis of comparison for the study on the bacteriology of acute diarrheas.

Progress:

Prior to the present Quarterly Report, 656 normal Thai individuals living in 3 communities near Bangkok had been cultured. Sixty two (9.5%) were found to harbor Salmonella species, and from 3 others (0.5%), Shigella were isolated. During the Quarter described in this report, stool specimens from an additional 810 persons were examined. One hundred twenty three (15.2%) yielded Salmonella, and from 6 (0.7%), Shigella were isolated. Many paracolon bacilli, including members of the Providence group, also were recovered from the stools examined. In addition, Proteus, Pseudomonas, and Bethesda-Ballerup bacilli were encountered frequently. Arizona bacilli were isolated on occasion.

• Among the individuals harboring Salmonella, there was one case from whom two different species were cultured. In another instances, a Salmonella and a Shigella species were isolated from a single stool specimen.

To date, of 1466 normal individuals examined, Salmonella have been isolated from 185 persons (12.6%), and Shigella from 9 (0.6%). Thirty different Salmonella species have been found so far, the most frequently isolated ones belonging to Groups B and E. Regarding the Shigella, 8 different species have been isolated.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01, Communicable Diseases (FIC 410)

Subtask: Enteric Infections

Study 1. Bacteriologic Survey of Stools from Normal
Individuals
(SEATO MEDIC Study No. 63)

Investigators: Lt. Colonel Sidney Gaines, Dr. Chiraphun
Duangmani

Description:

To provide information on the types of enteric pathogens found in the normal Thai population, and to provide a basis of comparison for the study on the bacteriology of acute diarrheas.

Progress:

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To date, of 1466 normal individuals examined, Salmonella have been isolated from 185 persons (12.6%), and Shigella from 9 (0.6%). Thirty different Salmonella species have been found so far, the most frequently isolated ones belonging to Groups B and E. Regarding the Shigella, 8 different species have been isolated.

Task 01 (Project No. 3A O 12501 A 806) Cont

The specimens tested were obtained from various age groups and from both sexes in each of the 3 communities under investigation. With respect to the nature of the communities, one is urban in character, another is rural, and the third is a combination of the two types.

Summary and Conclusions:

It is apparent that a significant portion of the normal Thai population harbors enteric pathogens, particularly the Salmonellae. Of particular interest is the wide variety of Salmonella species isolated. This study is continuing.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask: Enteric Infections

Study 2. Testing and Evaluation of a Holding Medium for
Enteric Bacteria. (SEATO MEDIC Study No. 64)

Investigators: Major Eugene B. Blair, Miss Sylvia Carey (WRAIR),
Lt. Colonel Sidney Gaines, Mr. Shamin ul Haque,
Mr. Wichian Panom

Description:

To determine how long a specially prepared holding medium inoculated with stool samples or rectal swabs will be successful in maintaining the viability of the enteric bacteria, particularly the pathogenic types, which may be present.

Progress:

To date 205 fecal specimens have been collected for examination in this study. Duplicate and in some instances triplicate swabs were taken from each stool and placed in either 2 or 3 vials of holding medium. One vial was sent to the Department of Bacteriology, WRAIR, for examination, the second vial was cultured in this laboratory (SMRL) 24 hours after the specimen was obtained, and if triplicate swabs had been taken, the third vial was cultured in this laboratory 1 week later. All vials were kept at room temperature at all times, including during shipment to WRAIR.

From the 205 fecal specimens collected in the holding medium and tested in this laboratory, 18 Salmonella, representing 11 different species, were isolated. In addition, 10 Shigella (4 species), 18 enteropathogenic Escherichia coli, and 20 Providence bacilli were cultured. Many other Enterobacteriaceae also were found.

The findings in this laboratory and those in the WRAIR laboratory have been kept separate, so as to avoid the introduction of possible bias.

Task 01 (Project No. 3A 0 12501 A 806) Cont

Summary and Conclusions:

Collection of specimens is continuing. Observations made so far in both laboratories indicate that the viability of the "normal" enteric flora is being maintained. A comparison of the findings in the two laboratories is planned for the near future, at which time an initial evaluation of the ability of this medium to maintain the viability of enteric pathogens can be made.

Department of Bacteriology & Immunology

General Information:

A. Water specimens for bacteriologic examination:

During the Quarter a total of 56 water samples were submitted to this laboratory for bacteriologic analysis. Two specimens were from swimming pools, and the remainder came from actual or potential sources of drinking water. The specimens were submitted by Thai and U.S. military and civilian agencies, and by individuals.

B. Klong waters:

Examination of klong (canal) waters from 10 different klongs in the Bangkok area for the presence of cholera vibrios continued. In no case were true cholera organisms isolated, although non-agglutinating vibrios were cultured from some of the samples.

C. Anthrax:

On 22 February 1963 smears taken from skin lesions of two patients suspected of having anthrax were brought to this laboratory for microscopic examination. Gram positive bacilli morphologically resembling Bacillus anthracis were seen. Similar findings were made on smears taken from cow meat, with which the patients had been in contact. These patients were hospitalized in Dhonburi, a section of Bangkok.

On 15 March 1963 blood from a Palm Civet, which had died in the zoo at Chiangmai in northern Thailand, was obtained for culture. A pure culture of a gram positive, sporulating bacillus was obtained. A guinea pig inoculated subcutaneously with a light suspension of the organisms died in 48 hours. Smears made from the spleen and heart blood showed large numbers of large, gram positive bacilli in chains, which resembled B. anthracis. The organisms were also cultured from the spleen and the blood, and showed a colony appearance typical for this bacillus. The probable source of infection in the Palm Civet was from contaminated meat from a water buffalo.

General Information (Bact Dept) Cont

D. Miscellaneous:

In support of various organizations and units, serologic or bacteriologic studies were performed on the following:

1. 1 serum specimen for Widal test from the 31st Field Hospital, Korat.
2. Sterility checks on media used by the Department of Veterinary Medicine, SMRL.
3. Examination of the premature and full-term sections of the Pediatrics Department of Chulalongkorn Hospital for possible contamination by enteric pathogens. Twelve areas were included in this examination.
4. 7 specimens of purulent material from liver abscesses to be examined for enteric bacteria submitted by the Faculty of Tropical Medicine, University of Medical Sciences.
5. 1 urethral discharge for microscopic examination and culture submitted by the JUSMAG Medical Unit.
6. 1 culture of an enteric organism submitted by the bacteriology laboratory of the Women's and Children's Hospital for identification.
7. 10 possible Salmonella cultures submitted by the Thai Army routine diagnostic laboratory for identification.
8. 4 stool specimens submitted from the RAAF Base at Ubol, Thailand.

In addition to the above, an autogenous vaccine of Micrococcus pyogenes, variety aureus (Staphylococcus aureus) was prepared.

Department of Entomology

Project No. 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Disease

Subtask: Arthropod-borne infections

Study 1. Arthropod-borne viruses in the Bangkok area
(SEATO MEDIC Study #40)

Investigators: Major John E. Scanlon, Miss Pacharee Nawarat*

* In the Quarterly Report for October-December 1962, Mrs. Rampa Rattana-rithikul was listed as investigator in this study in error.

Description:

Mosquitoes are collected from each of five areas of Bangkok, chosen in coordination with the Virus Department (SEATO MEDIC Study #7). Mosquitoes are collected by light traps and taken while resting in dwellings during the day. Additional specimens are taken while attempting to bite collectors during the daytime, indoors and near houses; and additional biting collections are made outdoors and indoors in the early hours of darkness. A net trap is operated, with the collector serving as an attractant; and an empty net trap is operated simultaneously as a control. Mosquitoes collected in a satisfactory condition are maintained alive long enough for digestion of their blood meal, then anaesthetized, identified and frozen for virus isolation attempts. Population trends for each species, by area and for the city as a whole, are plotted for comparison with the trend of arthropod-borne virus disease in the city.

Progress:

The routine collections from the five study areas were concluded in January, with the completion of a year of collecting. The statistical data by area and time were brought up to date in the succeeding weeks, while the collecting personnel were engaged in a special study of the mosquito population at Don Muang International Airport. A report on this activity is appended to the present report. In March, a routine collecting schedule was resumed in Bangkok, in two of the five areas used in the previous surveys. These areas were of special interest because of the number of cases of hemorrhagic fever detected there, and because of the time of appearance of the cases. Collections in these areas are limited to resting and biting collections as sources of specimens for virus isolation.

Task 01 (Project No. 3A O 12501 A 806) Cont.

During the Quarter an additional 4,740 mosquitoes were preserved for virus isolation. These consisted of 4442 Culex pipiens quinquefasciatus and 298 Aedes aegypti. All of the mosquitoes from the year-round study in Bangkok have now been turned over to the Virus Department for isolation. A total of 27 virus strains have now been isolated from material collected in the study. A discussion of these isolations will be found in the report of the Virus Department. No species were added to the Bangkok list during the Quarter, and it is believed that the list as it was reported in the last quarterly report. Studies have been initiated on several of the species complexes in the Bangkok area, by rearing of sibling series. It is expected that this technique will permit identification of some of the difficult species in the Bangkok collections, such as Anopheles barbirostris.

The weather conditions during the quarter were generally unfavorable for large numbers of mosquitoes. Minimum Temperatures were lower than in the preceding quarter, and lower than 20°C. on thirty one days during the period. The lowest nighttime temperature during the quarter was 11.6°C. No rainfall was recorded for January, and there was 10.0 mm. recorded in February. In March, all of the 40.0 mm. recorded fell in one day. The general trend therefore was toward the drying of mosquito breeding sites.

Project 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Disease

Subtask: Arthropod-borne infections

Study 2. Japanese B encephalitis at Bang Phra, Choburi
(SEATO MEDIC Study #41)

Investigators: Maj. J.E. Scanlon, Miss Pacharee Wawarat, Dr. Sakorn
Resitayodthin*, Mr. Choomporn Bhundhusoon*

Description:

Mosquitoes are collected at least three times a week at the Bang Phra laboratory of the Queen Saovabha Institute, in Southeastern Thailand. Parallel collections are made by means of a horse-baited large mosquito trap, and from a special light trap designed to capture live mosquitoes. The mosquitoes are anaesthetized and the common species used in virus isolation attempts. The less common species are sent to Bangkok for identification. Lists of all species and population data are maintained by this Department. Viruses isolated from the mosquitoes or from horses at Bang Phra are turned over to the Virus Department SMRL for additional study.

Progress:

From January to mid-March forty six (46) additional light trap collections were processed. The number of females per trap night showed a decrease in this Quarter, as expected during the cold dry months of January through March. The number of females per trap night in the horse baited trap, however, held rather steady. The number of females taken by the bait trap method was still quite small as compared with the light trap method. The overall average of females per trap night for the bait trap in this Quarter was 52.9, as compared with 54.7 in the preceeding Quarter. One additional isolation of a virus from Culex tritaeniorhynchus was reported during the period. However, the mosquitoes were collected in November, and all five of the virus isolations made thus far were from mosquitoes collected in October and November.

TABLE 1

Mosquito Captures at Bang Phra, Choburi, January-March 1963

MONTH	FEMALES PER TRAP NIGHT	
	<u>Light trap</u>	<u>Bait trap</u>
January	423.45	57.09
February	208.11	43.26
March (partrol)	228.40	58.60

* Bang Phra Laboratory, Queen Saovabha Institute

Task 01 (Project No. 3A 0 12501 A 806) Cont.

TABIE 2

Percentages of Culex gelidus and Culex tritaeniorhynchus in Bang Phra
Light trap and Bait Trap Collections January-March 1963

MONTH	LIGHT TRAP			BAIT TRAP		
	C.gelidus	C.tri.	Combined	C.gelidus	C. tri.	Combined
January	74.05%	16.04%	90.09%	91.48%	2.95%	94.43%
February	45.47%	12.01%	67.48%	64.11%	20.07%	84.18%
March	69.96%	19.79%	89.75%	82.59%	4.78%	87.37%

Summary and Conclusions:

Additional collections of mosquitoes were processed for virus isolation, and additional data were compiled on the seasonal distribution of mosquitoes in the study area. One additional strain of virus was isolated from Culex tritaeniorhynchus.

Project No. 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Disease

Subtask: Arthropod-borne infections

Study 3. Mosquito-borne viruses in other areas of Thailand
(SEATO Medic Study #42)

Investigators: Major J.E. Scanlon, SFC A.C. Fulmer, Mr. Kol Mongkolpanya
Mr. Sumeth Chunchulcherm

Description:

Mosquitoes are collected in various parts of Thailand in connection with studies of the mosquito fauna of the country, and in connection with investigations of human disease outbreaks. When possible, aliquots of these collections are frozen and turned over to the Virus Department for isolation. In addition several routine collections sites have been chosen, within reasonable driving distance of Bangkok. These sites represent a number of ecotypes and are surveyed on a monthly schedule, with emphasis on collections from human dwellings, animal and human biting collections.

Progress:

During March two study sites were selected, and routine collections were begun. Each site is visited at bi-weekly intervals by a team consisting of three collectors and a technician who identifies the most abundant species in the collections, seals them in ampoules and freezes them. The less abundant or difficult species are preserved for identification at the Bangkok laboratory. The areas visited thus far are a village at Rangsit on the rice plain near Bangkok. This village is of linear shape, along a main klong or canal. The other site being visited is Amphur Pakchong, in the foothills of the Petchabun mountain chain, northeast of Bangkok. U.S. Army troops were billeted in that area some months ago, and showed serological evidence of exposure to the arthropod-borne viruses. Mosquitoes from the collections still being processed. The collections have been quite small thus far, due to the extreme dryness of the study sites. Large number of mosquitoes will not be found until mid-May or later. Additional study sites will be added to this project when an evaluation of the efficiency of the collections is available, and when the seasonal changes in the Bangkok region indicate larger numbers of mosquitoes are occurring.

Summary and Conclusions:

Collections of mosquitoes for virus isolation are proceeding in selected ecotypes near Bangkok, and in other areas of Thailand as opportunity permits. Results of virus isolation are not available as yet.

Project No. 3A 0 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Disease

Subtask: Arthropod-borne infections

Study 4. Colonization of vector mosquito species
(SEATO MEDIC Study #43)

Investigators: Lt. James M. Neely, Miss Nantana Fuangarom
Miss Rachanee Dechasiri, Mr. Kol Mongkolpanya
Mr. Sumeth Chunchulcherm

Description:

Colonies of several important mosquito species are being maintained for projected virus and malaria transmission studies, and to serve as standards in insecticide resistance studies.

Progress:

Colonies of Culex pipiens quinquefasciatus and Aedes aegypti were maintained as usual during the period. Both were used for several tests of insecticides during the Quarter, and an initial attempt was made to transmit Chikungunya virus by the bite of Culex pipiens quinquefasciatus. Results of the latter experiment will be reported at a later date. The very small numbers of Anopheles minimus larvae collected in Southeast Thailand during February were not sufficient to permit establishment of a colony, and larger numbers were not found despite a concentrated search in an area known to produce large numbers during the rainy season. Field work is continuing in the area in order to detect changes in the population level of A. minimus as soon as the population begins to rise. A small source of larval and adult Anopheles balabacensis was found in the Chantaburi area in February, (SEATO Medic Study #86). Adults returned to the laboratory failed to lay eggs, and had a relatively short longevity. Many larvae were reared to the adult stage, and the identity of the larvae and adults checked. All were Anopheles balabacensis balabacensis, the nominate subspecies, as defined by Colless. The adults proved difficult to feed in the laboratory, and no fertile eggs have been obtained. It will be necessary to use the forced insemination technique to obtain a sufficient number of fertile eggs to assure perpetuation of the colony. Plans have been completed for expansion of the insectary facilities in the RTAIP building for specific use of the special project in malaria. Two members of the Department visited the Institute for Medical Research, Kuala Lumpur Malaya, for training in special insectary techniques in connection with malaria investigation. Special emphasis was placed on training in the artificial insemination technique.

Summary and Conclusion:

Colonies of several culicines were continued as usual, providing stock for virus transmission experiments and insecticide tests. Initial attempts were continued for colonization of several Anopheles species.

Project No. 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Disease

Subtask: Arthropod-borne infections

Study 5. Precipitin tests of mosquito blood meals
(SEATO MEDIC Study #44)

Investigators: SFC A.C. Fulmer, Miss Nantana Fuangarom

Description:

Engorged mosquitoes are removed from collections being processed for virus isolation and smeared on filter paper for later determination of the sources of the blood meal by the precipitin technique. Specific antisera to selected avian and mammalian hosts are prepared in rabbits.

Progress:

Two additional antisera were prepared during the Quarter, using the technique described previously. An antiserum to dog was prepared with a titer of 1:4000, with no cross reaction to any of the other hosts used in the series at that level. An antiserum to monkey prepared in February did not yield a satisfactory titer, but the preparation was repeated in March, resulting in antiserum which titered 1:8000, with no cross reaction to the other hosts used in the test. The complete range now used consists of the following hosts: human, horse, cow, pig, chicken, dog and monkey. It is hoped to extend the testing to Anopheles mosquitoes in the following period, in connection with studies on human malaria. From 28 December to 8 March, three hundred and thirty nine (339) tests of mosquito blood meals were completed. Beginning on 8 March the tests were expanded to include dog antiserum, and late in March to include monkey antiserum. All tests thus far have been restricted to the two most common species taken in studies of hemorrhagic fever in Bangkok, Culex pipiens quinquefasciatus and Aedes aegypti. Complete results of reaction to the full range of antisera are not yet available.

Summary and Conclusions:

Two additional antisera were prepared during the period, dog and monkey. Over three hundred blood meals were tested against part of the complete battery of antisera. Results are still incomplete.

Project No. 3A 0 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Diseases

Subtask: Arthropod-borne infections

Study 6. Association of Diptera with incidence of trachoma
(SEATO MEDIC Study #45)

Investigators: Maj. J.E. Scanlon, Mrs. Rampa Rattananarithikul,
Dr. Satma Singh*

Description:

Populations of eye gnats (Chloropidae) and filth flies (largely Muscidae and Calliphoridae) are studied in Korat city and three villages in the vicinity by means of two types of bait traps. These are operated by personnel of the WHO Trachoma Control Project and the catches are forwarded to this Department for counting and identification. The results of the population study will be examined in comparison with the incidence of trachoma in the selected villages through the year.

Progress:

All collections in the study area have now been separated and the field work has been suspended. Identifications of the aliquots of the collections have not as yet been received from the United States National Museum. A final report has been received on the relative abundance of trachoma cases in the study areas during the months in which flies were collected in the Korat area. These data were accumulated during monthly visits to the villages by WHO teams, and visual examination of all children under 10 years of age. These data will be correlated with the fly abundance figures when identifications are completed.

Summary and Conclusions:

Field work on the project, both fly collections and disease incidence studies have been completed. A final report will be prepared when identifications of aliquots of the fly collections have been received from the USNM.

* WHO Trachoma Control Project

Project 3 A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01: Communicable Diseases

Subtask: Rickettsial diseases

Study 1. Collection of chiggers and ticks for rickettsial isolation
(SEATO MEDIC Study #46)

Investigators: Capt. Vichai Sangkasuvana+, Maj. J.E. Scanlon, Mr. Kitti
Thonglongya, Maj. Samnieng Buspavanich+, Dr. Bennett
Elisberg*, Capt. Garrison Rapmund*, Dr. Robert Traub**

Description:

Larval trombiculid mites (chiggers and various stages of ticks are removed from their vertebrate hosts and forwarded to Bangkok, either frozen or in the living state, for attempts to isolate rickettsiae in appropriate laboratory animals. Tissues are recovered from mammals for additional isolation attempts, and as many animals as possible are bled for serological examination.

Progress:

Reports have been received from the investigators at WRAIR on the serological examination of human and other mammalian blood collected during the initial period of field work in Northern Thailand. These indicate the widespread occurrence of several rickettsial diseases among man and animals in all of the areas studied. Details of these serological results will be listed in future reports. Rickettsiae were isolated from rodents collected in several areas of Thailand, including sites near Chiangmai and Khon Kaen and from Chong Mek on the Eastern border of Thailand. Additional specimens of mammals and ectoparasites were made at Chantaburi in Southeast Thailand, at Pong Nam Rawn on the Cambodian border. Reports have not been received thus far on the isolation attempts using ticks collected during December and forwarded to WRAIR. The general trend of chigger populations has been decidedly downward in this Quarter, and several animals collected at various sites gave evidence of considerable earlier chigger attachment. Many of the specimens now collected in the field are being inoculated into mice at the collection site, with additional specimens of tissues being frozen for later study. Chiggers are now routinely inoculated in the field, as shipment to the central laboratory resulted in excessive death of the chiggers, or in their transformation to the protonymph stage. Study sites have been selected in the Chiangmai area, based on isolations made during the quarter, and these study sites will be examined in detail throughout the year. Additional study sites have been selected in the region within two hundred kilometers of Bangkok, and these will be surveyed from the Bangkok laboratory several times during the year. The principal study area under present consideration is in the Chong Mek region along the Mekong River, east of Ubol. Identification of all lines of rickettsiae isolated is still in progress.

** University of Maryland

* WRAIR

+ Royal Thai Army Medical Service

Task 01(Project 3A 0 12501 A 806) Cont.

Summary and Conclusions

Serological evidence has been obtained for the existence of several species of rickettsiae in Thailand. Isolations of rickettsiae have been made from mammals collected in several parts of the country. The isolation program is being continued in several areas, and sites for detailed examination have been selected.

Project No. 3A O 12501 A 306 MILITARY PREVENTIVE MEDICINE

Task 04: Ecology and Control of Disease Vectors and Reservoirs

Subtask: Arthropods of medical importance in overseas areas

Study 1. Mosquito fauna of Thailand
(SMATO MEDIC Study #47)

Investigators: Major J.E. Scanlon, Mrs. Rampa Rattanarithkul,
Mr. Sahem Esah, Miss Supanee Sandhinand

Description:

This study of the taxonomy and ecology of the mosquitoes of Thailand is **co**related with, and arises from, studies of mosquito-borne disease. Mosquitoes are collected routinely on a year-round schedule in Bangkok, Chiangmai, Udorn and Bang Phra. In addition, other collecting sites in many areas of Thailand are visited as often as possible. Distribution records, records of human and animal biting, breeding sites, and other facets of the biologies of the various species are recorded. Publication of a monograph on the mosquitoes of Thailand is the eventual goal of the project.

Progress:

During the Quarter the first shipments of mosquito specimens for illustration were sent to the 406th Medical General Laboratory. These consisted of females of the subgenera Anopheles and Celia of the genus Anopheles. Progress was also made in selection of specimens of other genera for illustration, and on arrangements for the final form of the publication. Contact was also made with other institutions which will make specimens available for illustration which are not presently in our collections. It is anticipated that completion of the illustrations of all Thailand mosquito species will require approximately two years. Several additional species were added to the Thai fauna during the period, including several species apparently new to science. Additional time was spent during February in study of the systematic collections at the Institute for Medical Research in Kuala Lumpur, Malaya. Arrangements were made there to borrow series of topotypic specimens for species occurring in Thailand.

Artificial breeding containers in the Chiangmai area have yielded many interesting specimens of several genera. These collections were made at a time when natural breeding sites were almost completely dry. It appears that breeding of some important genera, such as Tripteroides and Aedes (Finlaya) may continue through the dry period of the year in the artificial containers. Biting collections in the forests around Chiangmai have fallen off sharply during January through March, and collections from light traps and a cow-baited trap have also paralleled

Task 04 (Project No. 3A O 12501 A 806) Cont.

the fall in numbers of specimens biting man. Additional collections were made in the Chantaburi region, in connection with S IATO Medical Study #86, and these observations will be discussed in that section.

Summary and Conclusions:

Several new species have been added to the list of the mosquito fauna of Thailand. Illustrations have been begun for a projected monograph on the mosquitoes of the country.

Project No. 3A O 12501 A 306 MILITARY PREVENTIVE MEDICINE

Task 04: Ecology and Control Disease Vectors and Reservoirs

Subtask: Arthropods of medical importance in overseas areas

Study 2. Culicoides of Thailand
(SEATO MEDIC Study #48)

Investigators: Mrs. Rampa Rattanarithikul and Dr. W.W. Wirth*

Description:

In order to determine the distribution of these important Diptera in Thailand, specimens are removed from routine light trap and human biting collection whenever time permits. These are preserved in alcohol and sent to the United States National Museum for determination. These gnats transmit several human disease agents (Mansonella and Acanthocheilomena) and several important diseases of domestic animals (blue tongue virus and african horse-sickness virus). Their status as disease vectors and their distribution and biology in Thailand are virtually unknown.

Progress:

From January to March additional specimens of Culicoides were segregated from light trap collections from Bangkok, Bang Phra and Chiangmai. No additional shipments were made to The United States National Museum during the period, and the rate of collection has been slowed by the cessation of routine collections in the Bangkok area. Emphasis will now be placed on the rearing of Culicoides and other Heleidae encountered in routine collections of mosquito larvae. This method of collection has been requested by Dr. Wirth, in order to settle some of the complex taxonomic questions arising from the collection of adults only. Approximately two hundred vials of light trap material are now on hand for shipment. No additional identifications have been received from Washington during the period.

Summary and Conclusions:

Routine collection of Culicoides midges was continued during the Quarter. Collectors have been instructed to save midge pupae for rearing, and the collection of adults from light traps has been diminished.

* USDA, U.S. National Museum

Project No. 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 04: Ecology and Control of Disease Vectors and Reservoirs

Subtask: Arthropods of medical importance in overseas areas

Study 3. Ectoparasites of the vertebrates of Thailand
(SEATO MEDIC Study #49)

Investigators: Mr. Kitti Thonglongya, Miss Panita Lakshana, Mr. Inkam
Inlao, Mr. Preecha Ruangvija, Mr. Samarn Maneewongse

Description:

Ectoparasitic arthropods are removed from all birds, mammals and reptiles collected in connection with disease studies. These arthropods sorted for determination by the Department staff and cooperating specialists. The program is designed to provide a list of the medically important arthropods of Thailand, as a guide for future research; and to provide identification service for isolation programs presently in progress.

Progress:

Two Department personnel visited the Institute for Medical Research in Kuala Lumpur, Malaya in February for familiarization with the methods used there for mammal and ectoparasite identification. One technician received additional training in mammal identification with Dr. J. L. Harrison, Chairman of the Zoology Department, University of Singapore. All of the mammals collected prior to January 1963 have now been reexamined and the identifications checked. During the period five hundred and six skulls of mammals were cleaned and examined with their matching skins. At present the mammals in our collections represent seven orders, sixteen families, twenty seven genera and thirty seven species. Work is proceeding on subspecific identification of the hosts, particularly the rodents.

Collection of mammals was continued in the Chiangmai area, to determine the changes in ectoparasite fauna through the year. Mammals were collected at various altitudes on the surrounding mountains, and at two villages on the valley floor where scrub typhus has been known to occur. Mammals were also collected in the Chantaburi and Pong Nam Rawn areas of South-eastern Thailand. A list of the mammals collected in these areas is given in Table 1. Many of the rodents examined showed evidence of much heavier chigger infestation earlier, but the yield of chiggers at this season was quite small. A significant number of nasal chiggers was found, however. More fleas were collected from the mammals than had appeared in our earlier collections, and there were as many as 14 Xenopsylla cheopis per rat. The collection sites are described in SEATO Medic Study #86 of this report.

Task C4 (Project 3A O 12501 A 106) Cont.

TABLE 1

Animals and Ectoparasites from Southeastern Thailand

Name of Host	No. of Collected	No. of insected host	Lice	Flea	Tick	Mite	Chig- gers	Others
1. <i>Rattus rattus</i>	12	11	3	2	0	8	11	0
2. <i>Rattus rattus</i>	20	18	0	1	2	14	17	2 Pseudoscorpion
3. <i>Rattus berdmorei</i>	6	6	0	5	0	2	5	0
4. <i>Bandicota indica</i>	2	2	0	1	0	0	1	1 Malaria
5. <i>Menetes berdmorei</i>	26	23	13	4	3	10	17	2 Malaria 1 Adult filarial worm 2 Microfilaria.
6. <i>Tupaia glis</i>	2	2	0	1	2	2	2	1 Microfilaria. 1 Malaria
7. <i>Melogale personata</i>	2	0	0	0	0	0	0	0
3 Orders, 4 Families, 5 genera 7 species	70	62	16	14	7	36	53	10

Determinations for material collected in this and preceeding Quarters are as follows:

a. Chiggers - (Miss Panita Lakshana, Mr. Kittu Thonglongya) Preliminary identifications are still made in the field, before inoculation of mice, and aliquots of these inoculation type specimens are confirmed in the laboratory. Approximately eighty species of chiggers are now represented in our collections, of which twenty are new species. Samples of the latter have been sent to the 406th Medical General Laboratory in Japan for illustration, and to Dr. Traub for preparation of diagnoses and descriptions. The status of the species complex centered around Leptotrombidium deliensis is still under investigation. Variations detected in specimens identified as L. deliensis indicate that the species is either exceptionally variable, or that a series of sibling species is involved. Examination of specimens in the collections of the Institute for Medical Research in Kuala Lumpur, indicates that many of the Thai species of chiggers are also found in Burma, Laos, Vietnam and Malaya. The new species which are currently being drawn and described include the following: Three species near Leptotrombidium deliensis; eight other species of Leptotrombidium and one species of Gahrlepieia. Two additional slide preparators have been engaged in order to speed the identification of the chiggers and the preparation of material for description.

Task 04 (Project No. 3A O 12501 A 806) Cont.

b. Hippoboscidae and other Pupipara: Two reports have been received from Dr. T.C. Naa, Taiwan, containing identifications of pupiparous Diptera from mammals and birds in our collections. These include several new species.

c. Other ectoparasites: Additional Anoplura from Thailand, and specimens submitted from Pakistan have been identified in this Department. No additional reports of identifications have been received from cooperating agencies during the quarter.

Summary and Conclusions:

Approximately two hundred additional vertebrates were examined from Northern and Southeastern Thailand during the quarter. Considerable progress has been made on the description of new species of chiggers and on the cataloging of all of the ectoparasites of Thailand.

Project No. 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 04: Ecology and Control of Disease Vectors and Reservoirs

Subtask: Insecticide Tolerance of mosquitoes

Study 1. Determination of insecticide Tolerance level of selected mosquito species in Thailand
(SEATO MEDIC Study "50)

Investigator: Lt. Neely, Mr. Kol Mongkolpanya

Description:

The insecticide tolerance levels of the most important species of mosquito in Thailand are being determined by means of the World Health Organization test kits, as recommended by the Armed Forces Pest Control Board. Initial emphasis has been placed on the study of the most important adult species in the Bangkok area, with a planned later extension to other populations. Additional studies will be conducted with the WHO larval test kit.

Progress:

Additional checks we made on the adult mosquitoes from our laboratory colonies of Culex pipiens quinquefasciatus and A. aegypti, both of Bangkok origin. Culex pipiens quinquefasciatus with DDT yielded an LC-50 of approximately 0.5% and an LC-90 of 2.0%. A. aegypti with DDT yielded LC-50 of 1.0% and an LC-90 of approximately 3.0%. The same mosquitoes when exposed to Dieldrin resulted in an LC-50 of 1.6% for Culex pipiens, and an LC-50 of 0.1% for A. aegypti. Laboratory and wild populations showed similar results in both cases. The larvae of both Culex pipiens and A. aegypti were also tested and yield a LC-50 of 0.10% DDT for Culex pipiens quinquefasciatus and a LC-50 of 0.50% for A. aegypti. These results were comparable with those from wild populations. Culex pipiens quinquefasciatus larvae were also exposed to BHC resulting in a LC-50 of 0.03% and a LC-90 of 0.50%

The only noticeable resistance detected in these tests was with Culex pipiens quinquefasciatus. The LC-50 was 1.6% the highest concentration in the WHO kit for dieldrin, an indication that Culex pipiens quinquefasciatus has developed resistance to this particular insecticide. Although no general mosquito control program by the government has taken place in the Bangkok area, it is believed that the use of a local product, consisting of dieldrin, by the people in large amounts might be producing some tolerance. More detailed studies with this mosquito plus others will take place at a future date.

Summary and Conclusion:

Tests of the tolerance of laboratory and wild strains of Culex pipiens quinquefasciatus and Aedes aegypti were continued using several chlorinated hydro carbon insecticide. Tolerance of adult C. pipiens quinquefasciatus to dieldrin was detected and is being investigated further.

Project 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01 Communicable Diseases

Subtask ag. Arthropod-borne infections

Study 7. Anopheles in relation to malaria. (SEATO MEDIC Study # 51)

Investigators: Dr. Udaya Sandhinand, Maj.J.E. Scanlon, Lt.J.M. Neely,
Mr. Sahem Esah

Description:

Anopheles mosquitoes are collected by various methods from study sites selected for high incidence of malaria. The mosquitoes are dissected to determine the rate of infection with plasmodia. Plasmodia recovered are inoculated into experimental animals and human volunteers for recovery of strains of Plasmodium. Additional mosquitoes are collected for colonization for transmission experiments, and reared specimens are retained for feeding on gametocyte carriers to determine Anopheles species susceptibility.

Progress:

Two Department personnel were trained in mosquito dissection technique and allied subjects at the Institute for Medical Research, Kuala Lumpur, Malaya in February. In late February field work was begun at Chantaburi and Pong Nam Rawn in Southeastern Thailand. In Chantaburi area collections of adult and larval Anopheles balabacensis were made, and the specimens collected were returned to Bangkok for examination. The collections were made in the Tha-Mai area, 18 kilometers southeast of Chantaburi, along the coast, with large rubber plantations on the higher ground. The A. balabacensis were found under light shade in pits dug by workers mining sapphires and other gems. This larval habitat is somewhat different from that reported from other areas. Adults were also found resting on the walls of the pits. None of the mosquitoes dissected in the area showed evidence of infection. It is anticipated that the Tha-Mai region will produce large numbers of A. balabacensis during the rainy season. Larvae were also found along a small stream at the edge of the sapphire pits.

The Pong Nam Rawn area is approximately 45 kilometers northeast of Chantaburi, along the Cambodian border. It is a region of undulating hills and tall deciduous forests which have been heavily cut over, producing open glades of grassland (lalang). The area is reported to be highly malarious. Mosquito collections were poor in the area at the time of our visit, due to extreme dryness and low temperatures. Larvae were collected from large streams in small numbers, including A. minimus.

Task 01 (Project 3A 0 12501 A 806) cont.

However the small feeder streams where most of the breeding takes place were dry. Further collections will be attempted in the area after the onset of the rainy season, if air transportation is available. The road to the Pong Nam Tawn area is not passable during the rainy season. Additional collections of A. balabacensis can be made through out the year in the Tha-Mai area, which is served by an all-weather road.

Summary and Conclusions:

Field studies of anopheline population and collections for experimental work were begun in Southeast Thailand. An unusual breeding site for *Anopheles balabacensis* was detected. Collections were small at this period because of adverse weather.

Project 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01 Communicable Diseases

Subtask ag. Arthropod-borne infections

Study 8. Epidemiology of malaria. (SEATO MEDIC Study # 52)

Investigators: Dr. Udaya Sandhinand, Lt.Col. Kawee Suwankul

Description:

Areas of high incidence of malaria, or of unusual interest to the military are selected for epidemiological investigation. Longitudinal studies of infection in local populations and troop concentrations will be coupled with studies of selected cases in provincial and military hospitals. Special attention is given to detection of cases which apparently resist treatment by standard drug therapy.

Progress:

The Surgeons General of the Royal Thai Army, Navy and Air Force have requested subordinate medical commanders to report cases of suspected drug resistance to this laboratory. In addition plans have been made to establish clinical surveillance at several hospitals, civilian and military, where unusually large numbers of malaria cases occur. Initial blood film surveys were made in February in areas of known high endemicity. At Pong Nam Rawn, Chantaburi, thirty four persons were examined in house to house visits among the ninety seven houses of the village. Seven positive smears were found, all Plasmodium falciparum. A group of Royal Thai Marines stationed in the nearby jungle had arrived since the end of the rainy season and had experienced no malaria. However, the previous group, which had been in the area during the rainy season had a high attack rate, necessitating the evacuation of a number of men. In the Tha-Mai area, Chantaburi, seventy three school children were examined. Fourteen were positive for falciparum malaria, one for vivax. All of the children were healthy and attending school and are rated as a symptomatic parasitemias.

Summary and Conclusions:

Preliminary arrangements have been made for detection of foci of malaria, and field studies were initiated on a small scale during the period.

Project 3A O 12501 A 806 MILITARY PREVENTIVE MEDICINE

Task 01 Communicable Diseases

Subtask ag. Arthropod-borne infections

Study 9. Drug resistant malaria. (SEATO MEDIC Study # 53)

Investigators: Capt. Payong Tantinikorn, Dr. Udaya Sandhinand

Description:

A surveillance system is being established among military and civilian treatment facilities for detection of supposed cases of drug resistance in malaria cases. These cases will be investigated, and when positive smears indicate possible resistant malaria samples of blood and urine will be taken for biochemical determination. The area of probable infection will be determined for these cases, as a guide to selection of areas for further field studies. When drug resistance is proven by biochemical **procedures**, additional clinical and laboratory studies will be conducted.

Progress:

The laboratory equipment for drug determinations in blood and urine have been received from WRAIR, and are presently being installed and tested. Drugs and other chemicals are on order and should be available early in the following quarter. A surveillance system has been discussed with the military and civil health authorities and is presently being implemented.

Summary and Conclusions:

Initial work has begun on finding cases of presumed drug resistant malaria and on clinical and laboratory study of such cases.

Department of Medical Zoology

Project Number 34-0-12501-A-386, Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask a1: Parasitic Diseases. Studies on Opisthorchis viverrini in Thailand.

Study 1: Incidence of Opisthorchiasis in Remote Villages
(CMATO : FIC Study # 20)

Investigators: Major Carl E. Wykoff, Jr. (First Lieutenant (Medical School Drop Out),
Prof. Chanting Narinawata (Dean, First Lieut. School Drop Out), 1st Lt. L. L. Winn

Description:

The purpose of this investigation is to ascertain the prevalence of intestinal and hepatic parasites harbored by villagers in remote areas of North East Thailand.

Progress:

Since 1 January 1963, this study has been continued in 5 new villages in remote north east Thailand.

SUMMARY OF PARASITIC INCIDENCE IN SELECTED NORTH-EAST THAI VILLAGES

1 January - 31 March 1963	%	Total to Date	%
No. persons examined:	1716	4796	
No. with parasites:	1417	3563	33
No. with helminths:	1335	3366	30
No. with protozoa:	223	150	20
<u>Opisthorchis</u>	1290	3032	77
<u>Hookworm</u>	212	743	13
<u>Strongyloides</u>	143	453	10
<u>Taenia</u>	43	128	3
<u>Whipworm</u>	3	10	-
<u>Ascaris</u>	4	16	-
<u>E. histolytica</u>	3	40	1
<u>E. coli</u>	113	363	9
<u>E. nana</u>	35	310	7
<u>Giardia</u>	110	236	7

Pinworm examinations (Scotch Tape):

Children - age 0 to 6.....	exams...102....	26 positive (25%)
Children - age 7 to 12.....	exams....93....	15 positive (16%)

The age-sex data concerning the prevalence of Opisthorchis viverrini in villages Ban Nong Sai, Ban Kao Hoi, Ban Lam, Ban Nong N. Khem, Ban Nong No, Ban Nong Phai are presented in Table I. It is understood that the percentages in some cases are without meaning because of the small sample size and they should be read with this limitation in mind.

TABLE I.
AGE-SEX DISTRIBUTION OF OPTISTHORCHIS INFESTED IN SIX REMOTE
NORTH LAO VILLAGES

AGE	MALES			FEMALES			TOTAL		
	No.	Pos.	% Pos.	No.	Pos.	% Pos.	No.	Pos.	% Pos.
0-5	344	114	33	330	101	31	674	215	32
6-10	332	204	61	270	239	89	602	443	74
11-15	253	230	90	253	234	93	506	464	91
16-20	179	160	89	240	214	89	419	374	89
21-25	143	133	93	155	132	85	298	265	89
26-30	133	120	89	160	140	88	293	260	89
31-35	104	97	93	91	33	36	195	130	67
36-40	94	35	37	104	93	89	198	128	65
41-45	74	63	85	57	50	88	131	113	86
46-50	70	30	43	66	60	91	136	90	66
51-55	35	32	91	37	32	87	72	64	89
56-60	40	38	95	47	42	89	87	80	92
61-65	21	20	95	12	11	92	33	31	94
66-70	15	14	93	10	8	80	25	22	88
Over 70	15	14	93	17	15	88	32	29	91

Excluding the 0-5 year group the average % is 87

" " 6-10 " " " " % is 90

Summary and Conclusions:

The work will continue in new areas during the next quarter. The prevalence of Opisthorchis remains uniformly high. On the basis of very limited data gathered there appears to be no significant difference between the prevalence of infection in males and females. Now that the rice has been harvested, a larger percentage of middle-aged adults will be seen in the villages.

Project Number JA-0-00001-A-306, Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask a1: Parasitic Diseases. Studies on Opisthorchis viverrini in Thailand

Study 2: Snail Intermediate Hosts
(USATC MEDIC Study # 21)

Investigators: Mr Chamlong Surinsakul (Bangkok Cen. Hosp. Lab), Major Dale E. Wykoff
Dr Jostri Savakanta (Th. Sch. Trop. Med.), IS, Lt Col L. Winn

Description:

The purpose of this study is to determine which species of snail is the first intermediate host of opisthorchiasis in Thailand.

Progress:

The snail species mentioned in previous Quarterly Reports has been identified as Pithynia (Eulinia) (Allocinnia) (Eugoniostoma) p. niochhalus (Morelet 1863) with generic misspellings of Pithinia, Pythinia, and itinia. In addition another species, smaller when adult with a peculiar sinuous outer lip has been identified by Dr Morrison (US Nat. Museum) as Mattebledia crossiana (Mattebled 1934). The snail collections terminated with the onset of the dry season. Further work on the identification of cercariae and additional collections of snails must await the beginning of the rainy period. At the present there are no surface pools or streams in or around Udon, Thailand and water is temporarily a valuable commodity.

Summary and Conclusions:

The work will begin again when the first rain falls.

Project Number 3A-O-14501-A-306, Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask 01: Parasitic Diseases. Studies on Cryptothorax viverrini in Thailand

Study 3: The Fish Intermediate Hosts
(SLATO L.I.C. Study # 22)

Investigators: Dr Phat Pattijarat (U.S. Sch Trop Med), Major Dale E Nykoff,
Rgt Mar J Winn

Description:

The purpose of this study is to collect, identify, examine for metacercariae of all types and determine the average number of Cryptothorax viverrini in fish from different parts of Thailand.

Progress:

Summarized in Table II are the type and numbers of fish examined during this period, together with associated data.

Summary and Conclusions:

Of 1531 fish examined from Sakol Nakhon, 72% of Sambusia discus proved to be infected with metacercariae of C. viverrini, an average of 20 being found in each fish. While only 30% of the Pila landong were positive, they harbored on average of 105 metacercariae per fish. None of the 210 fish from Bangkok were positive. A total of 1741 fish were examined this quarter. The work will be continued.

TABLE II.
EXAMINATION OF FRESH WATER FISH FROM THAILAND FOR LETHAL DOSE OF CPISHTOCALIS

Scientific Name:	Common Name:	Number Examined:	Obtained From:	With O.v. No. %	Ave No. Lethal per Fish
Being determined	Pla Ma Long	46	Chabol Makorn Lake	2 4.34	0.23
Being determined	Pla Tog	255	"	139 50.42	105.17
Being determined	Pla Paasadang	133	"	67 50.37	16.15
Being determined	Pla Jaw	154	"	17 10.36	0.61
Puntius gonionotus	Pla Dra Pein Kau	34	"	1 2.941	2.52
Harpula dispar	Pla Saud	135	"	125 92.59	20.41
Puntiolites procyron	Pla Garong	112	"	3 3.04	0.14
Osteochilus haseltii	Pla Soi Nok Kau	100	"	- 4.34	0.23
Puntius orphoides	Pla Gam Cham	99	"	27 27.27	1.25
Puntius philopetorus	Pla Kowkeang	33	"	- -	-
Labioblabrus	Pla Sau Sai	23	"	1 3.57	0.21
Being determined	Pla Drapien Sai	134	"	37 35.57	1.96
Being determined	Pla Soi Kriep Daeng	77	"	- -	-
	Pla Soi Kriep Kau	18	"	- -	-
Being determined	Pla Sua	23	"	- -	-
Being determined	Pla Chalad	5	"	- -	-
Puntius viehovi	Pla Drapien	100	"	7 7	1.35
Harpula dispar	Pla Saud	2	Bangkok	- -	-
Osteochilus haseltii	Pla Soi Nok Kau	32	"	- -	-

TABLE II Continued

Scientific Name:	Common Name:	Number examined	Observed From:	With C.v. No.	%	Ave No. mites per fish
<i>Puntius vachao-</i> <i>veri</i>	Pla Bropien	75	Boe Rok	-	-	-
Being determined	Pla Lengdang	17	"	-	-	-
Being determined	Pla New Thai	17	"	-	-	-
Being determined	Pla Ma Yang	4	"	-	-	-
Being determined	Pla Hua On	5	"	-	-	-
Being determined	Pla Kru Lee	5	"	-	-	-
Being determined	Pla Chalad	16	"	-	-	-
<i>Punctiophites</i> <i>protogyrus</i>	Pla Sarang	13	"	-	-	-
<i>Labiobarbus</i>	Pla Sam Lai	26	"	-	-	-

Project Number SA-6-12501-A-306, Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask 01: Parasitic Diseases. Studies on Coelomorphis viverrini in Thailand

Study 4: The Animal Reservoir Hosts
(S. ATC MEDIC Study # 23)

Investigators: Dr Sastri Sompakant (Bkk Trop Lab), Major John L Wykoff,
Dr Chalong Karinasuta (Bkk Trop Lab), 1 Sgt Max L Winn.

Description:

This study is to provide information on (1) which animals are natural reservoir hosts of C. viverrini (2) ectoparasites for the Department of Entomology (3) blood specimens for the Virology Department (4) information on the species and distribution of animals in North East Thailand (5) kidney and liver sections for Leptospi-
ra investigations, and (6) to provide preserved mounted skins for the U.S. National Museum.

Progress:

During the period of this report 118 animals and birds have been trapped in the area within 40 miles of Udon Thani. Identification of the trapped species has not been completed but will be included in the next report. The types obtained were: rats (60), birds (27), bandicoots (27), pangolin (1), palm civet (1), monkey (1), flying squirrel (1)

Although dogs and cats are usually infected with O. viverrini, none have been found in the trapped animals.

Summary and Conclusions:

The work will be continued along the lines indicated above.

Project Number SA-O-12501-A-006, Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask a1: Parasitic Diseases. Studies on Opisthorchis viverrini in Thailand

Study 5: Experimental Laboratory Hosts
(STATO MEDIC Study # 24)

Investigators: Major Dale L. Wykoff, Jr Pilot Juthijudata (Bk. Sch Trop Med),
Dr Chamlong Parinasuta (Bk. Sch Trop Med), 1 Capt Max H. Winn

Description:

The purpose of this study is to collect basic data associated with the experimental infection of certain laboratory animals.

Progress:

Hamsters were experimentally infected with metacercariae of O. viverrini and data were collected on the prepatent period, number of metacercariae developing to adults, average number of eggs per gram feces per day and average number of eggs per worm per day. These data are summarized in Table II.

Summary and Conclusions:

The prepatent period in hamsters averaged 24 days, all observations being remarkable uniform with the exception of hamster number one which passed a single egg on day 14 but passed no further demonstrable eggs until day 24. The observation on day 14 may have been due to technical error. The percentage of administered metacercariae subsequently developing to adult worms in the biliary passages ranged from 40 to 94 (mean 70%). Animals were given three different dosages of metacercariae (50, 100 and 200) and the percent development to adult by group was 51, 80 and 69%, respectively. For these same groups, the average number of eggs per worm per day were, respectively, 39, 18, and 4. While data from these very small samples are of limited value, nevertheless the numbers of eggs per worm per day are extremely small, and there appears to be some indication that larger numbers of worms in the small hamster livers tend to produce fewer eggs. When sacrificed, these animals, even number 18, revealed the presence of numerous living adult worms containing normal-looking eggs. Further studies are to be made with hamsters both to increase the number of animals in each test group, and to attempt to determine why so few eggs are passed in the feces. Paralled studies will be made in the rabbit, an animal with a considerably larger liver. At the present it is difficult to obtain large numbers of metacercariae from fish and it may be necessary to wait for the start of the next rainy season before the new studies can be undertaken.

TABLE II.

EXPERIMENTAL INFECTION OF MICE WITH METACERCARIAE
OF *CHISTOCOLIS VIVERRINI*

Animal No.	No. of metacercariae	Prevalent period (da)	Days after infection when sacrificed	No. Adult worms recovered	% metacercariae developing to adults	Average E/GF/D*	Average GF/D**	Average total daily egg output per gram	Average No. E/G/D***
1	50	14	30	20	40	N/C	N/C	N/C	N/C
2	50	23	30	24	43	N/C	N/C	N/C	N/C
3	50	22	45	23	45	2667	0.6696	1768	78
4	50	25	1	-	-	-	-	-	-
5	50	25	60	34	68	3276	1.0243	5369	99
6	50	25	1	-	-	-	-	-	-
7	100	25	30	94	94	N/C	N/C	N/C	N/C
8	100	25	30	52	52	N/C	N/C	N/C	N/C
9	100	25	45	54	64	1653	1.0632	1763	23
10	100	25	45	33	33	2085	0.7790	1624	20
11	100	25	50	97	97	804	1.0542	348	9
12	100	25	60	87	37	1452	0.8792	1276	15
13	200	24	30	117	59	N/C	N/C	N/C	N/C
14	200	24	30	139	69	N/C	N/C	N/C	N/C
15	200	22	45	155	73	1238	0.3066	993	6
16	200	24	45	135	68	585	0.3445	573	6
17	200	24	60	130	65	701	0.3230	577	4
18	200	24	60	149	75	203	0.3592	174	1

E: Animal escaped

* Eggs per gram feces per day. Average of 15 counts, made 3 times daily for the five days preceding date of sacrifice.

** Grams feces per day. Average total daily fecal output measured daily for the five days preceding date of sacrifice

*** Average number of eggs per worm per day

N/C: Not counted: duration of infection too short.

Project Number 3A-0-12501-A-306, Preventive Medicine

Task 01: Communicable Diseases (TIC 410)

Subtask a1: Parasitic Diseases. Studies on Opisthorchis viverrini in Thailand.

Study 6: Morphology of Opisthorchis viverrini
(STATO MEDIC Study # 25)

Investigators: Dr Chamlong Larinasuta (Bkk Sch Trop Med), Dr Sastri Sawakonta
(Bkk Sch Trop Med), Major Dale E Wykoff, MSgt Max H Winn

Description:

This study is to examine the characteristics of the adult parasite on which specific differentiation is based.

Progress:

Adult worm specimens from a human autopsy have been stained and are being characterized according to the main morphological differences between O. viverrini and O. felinus. Camera lucida drawings are being prepared to show the variations in O. viverrini of the tests, ovary, vitelline, position of tests and cecum, and the shape of the seminal vesicle. Stained adult preparations sent to the U.S.A. and to Germany have not yet been morphologically studied and returned to us.

Summary and Conclusions:

During the past 3 months only one autopsy has been performed in Udorn Thani. A new morgue/autopsy room is being built. When such facilities as water and adequate lighting are installed it is anticipated that greater access to more autopsy material will be possible.

Project Number 3A-O-12501-A-006, Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask 01: Parasitic Diseases. Studies on Opisthorchis viverrini in Thailand

Study 7: Clinical Manifestations of Opisthorchiasis
(SATO MEDIC Study # 26)

Investigators: Dr. Suvajira Vajrasthira (Bkk Sch Trop Med), Major Dale E Wykoff,
Dr. Chamlong Paritsart (Bkk Sch Trop Med), Dr. Sastri Samkontha
(Bkk Sch Trop Med), 1 Sgt John L. Winn

Description:

Over 90% of the adult population in the Udorn area harbor hepatic trematodes and it would not be surprising to find certain clinical symptoms and syndromes associated with this infection. Ascites, edema, enlargement of the collateral circulation and of the abdomen, gastro-intestinal disturbances, anorexia, malaise and cachexia are believed to be caused by this parasite. However, only few data have been gathered on the specific relationship of the symptoms to the presence of the parasite. This study is being undertaken to determine whether Opisthorchis causes a specific disease, and if so to define its nature.

Progress:

At the Provincial Hospital, Udorn Thani (Dr. Kasem, Director), all patients are examined by one of our staff (Dr. Sastri) and the following information is recorded on a card prepared for this purpose - name, age, sex, address, ethnic background (Thai, Thai-Lao, Thai-Vietnam, etc), occupation, symptoms, results of physical, stool, blood count and blood chemistry (liver function) examinations. These data will subsequently be transferred to IDL cards. At the present some 60 patients are being studied weekly. The results are not yet ready for this report.

Summary and Conclusions:

The work will be continued in the Udorn Provincial Hospital as outlined above.

Department of Medical Zoology

General Information

During the past quarter, Udon experienced the coldest period of the year, the subsequent return of summer temperatures and the onset of the extremely dry season. In early February the temperature dropped to 37°F but by the middle of March the daily temperature ranged from 32° to 93° F. Almost all ponds and streams are completely dry and very few snails are to be found. Perhaps because there are no rains to carry the human feces into the few remaining ponds, the percentage of infected fish and numbers of metacercariae per fish are both low. Further studies requiring metacercariae and cercariae must await the start of the rainy season. Cooperation with the Provincial Hospital has been excellent and through the efforts of Prof Chamlong, a new wing is being built by the Thai Government on the hospital laboratory. This wing when completed will house the joint Bangkok School of Tropical Medicine - US Army SEATO Medical Research Laboratory Team in Udon. During March, at the request of the Prime Minister and through Dr Chamlong as Secretary General of the University of Medical Sciences, our combined team consisting of 21 persons participated for 18 days in the Rural Development Program in remote villages of the North East near the Thai-Lao border. The results of these studies will be reported in the next Quarterly Report.

Department of Pathology

Project No. 3A O 12501 A 806 Military Preventive Medicine

Task 01: Communicable Diseases (FIC 410)

Subtask: as Zoonotic Diseases

Study 1. Pathological survey of vertebrate fauna trapped in Udorn and Chiangmai regions. (SEATO MEDIC Study #90)

Investigators: Captain Sylvanus W. Nye, USAF, MC

Description:

Animals are collected in Udorn and Chiangmai regions by the Departments of Medical Zoology and Entomology. At field stations these animals are at present being studied for intestinal and ecto-parasites. At the time of these examinations portions of the viscera will be fixed in formalin and returned to Bangkok for histo-pathologic study and for correlation.

Progress:

Equipment has been collected for shipment to Udorn and Chiangmai.

Summary and Conclusions:

None

Department of Pathology

General Description of Activities:

The Department of Pathology began to function 29 January, 1963 with arrival of Captain Sylvanus W. Nye, USAF, MC, as chief of the department. The month of February was spent in becoming acquainted with the activities and procedures of the SEATO laboratories and the department of pathology of the Thai Army Institute of Pathology.

Some autopsies and surgical pathology specimens were examined during that time. The equipment for the histo-pathology began to arrive during February and has continued to arrive throughout the quarter. All the equipment is still not present in Bangkok. During March improvements were made in the Thai army histo-pathology laboratory with equipment which was being received.

From 11 March to 20 March the laboratory was visited by Drs. Robert E. Stowell and Chapman H. Binford, who visited with the express purpose of assisting in the establishment of projects in the pathology department.

During the last part of the quarter an application for a research project entitled: "Pathological survey of vertebrate fauna in the Udorn and Chiangmai regions", was prepared. Preparations were made for collections and assemblage of equipment for this project.

Department of Veterinary Medicine

Project No. 3A O 12501 A 806, Preventive Medicine

Task 01: Communicable Disease Military (FIC 410)

Subtask as: Zoonoses of military importance

Study 1. Isolation of Leptospires from Thailand
(SEATO MEDIC Study # 80)

Investigators: Captain Thomas J. Keefe, VC., and Dr. Prem Brahmacharya

Description:

To isolate as many strains of leptospires as possible from Thailand; and to examine ecological, climatic, or other variants determining the specific infections.

Components of study:

The study is composed of two main components:

A. Rodent trapping and culturing of animal tissues for leptospire organisms

B. Canal water inoculation from Thai Farm for recovery of leptospires

Study A.

Number of rodent kidneys cultured 121

Number of leptospire isolates 8

% isolates 6.6

No. 1 st cultures		% Contamination Tenbrook-Pipette		Darkfield suspect	No. isolates	
121		23	11	55	8	
Samples Inoc.		Die	Cultured	Cultured	Hamsters cultured	Cultured
Hamsters		6-21 day	-	-	at 30 days	30 day
48		2	2	2 0	8	7 neg
		#207			dkfld suspect	1 not mature
Second Pass.		Die	Cultured		Cultured	
Hamsters		6-21 day			- +	
43		0	2		2	

Task 01 (Project No. 3A 0 12501 A 806) Cont

No. 1 ^o cultures	% Contamination Tenbrook-Pipete	Darkfield suspect	No. isolates
121	23 11	55	8
Third Pass. Hamsters	Die 6-21 day	Cultured	
5	0	0	

Throughout the greatest portion of this quarter, hamster passage was done on rodent kidney grindings only if the kidney was designated "darkfield suspect" and hamsters were available for passage. Four isolates were not designated "darkfield suspect" upon original kidney grinding, and were therefore not put into hamsters. Two isolates (#207, #216) caused sickness and death in hamsters within 6-21 days, but non-contaminated Fletcher's media cultures (Tenbrook and Pasteur pipete) failed to grow the organism. Two isolates have not caused death in first passage hamsters at 20 days. This data indicates that darkfield examinations are not accurate indicators of the presence of leptospire. Our limited experience with hamster inoculation does not warrant comparative judgement of this route of isolation at the present time.

Buffalo and cattle kidneys have been picked up at the Bangkok abbatoir for primary culturing and hamster inoculation. Specimens were procured from baskets full of kidneys placed in a cold storage room of the abbatoir. Kidney capsules were stripped in a sterile manner, and kidney plugs removed with a sterile Pasteur pipete. These plugs were:

1. Inoculated into Fletcher's media - one tube
2. Ground with Tenbrook and inoculated into Fletcher's media - one tube
3. Tenbrook grinding inoculated into weanling hamster

CATTLE

No. 1 ^o cultures	Percent Contamination		Darkfield Suspect	Cultures Matured	Hamsters Innoculated
129	11		45	96 neg.	45
1st Pass. Hamsters	Die 1-5 da.	Die 6-21 da.	Darkfield Suspect	Cultures Innoc.	Hamsters 30 days & passed
45	5	0	0	0	30
2nd Pass. Hamsters	Die 1-5 day	Die 6-21 da.	Matured 30 da.		
30	0	0	0		

Task 01 (Project No. 3A 0 12501 A 806) Cont

BUFFALO

No. 1 ^o cultures	Percent Contamination	Darkfield Suspect	Cultures Matured	Hamsters Innoculated
	15	29	86 BCA 10 +	29
1 st Pass. Hamsters	Die 1-5 day	Die 6-21 da.	Darkfield Suspect	Cultures Innoc.
29	2	0	0	0
2 nd Pass. Hamsters	Die 1-5 da.	Die 6-21 da.	Cultured	Hamsters 30 days & passed
21	0	0	0	0

BCA 10 was innoculated into hamster originally on 19 Feb. Hamster was dead and discarded on 20 Feb.

It is evident that the method of collection of buffalo and cattle kidneys allows for excess contamination. In the future, an attempt will be made to procure the specimen in situ on the slaughter line.

Study B.

Canal water innoculation from Thai Farm for leptospire isolation

Surface water samples are collected from two sites of effluent drainage (part of a canal system) from the large swine farm. These are concentrated to 5 cc. aliquots by filtration, and innoculated 1 cc. into each of five weanling hamsters. On a series of 69 water samples, the following data was obtained:

# Samples	1 st Pass. Hamsters	Die 1-5 day	Die 6-21 day	Cultures Matured	Cultures +	Cultures -
69	345	11	12	9	0	9
2 nd Pass. Hamsters	Die 1-5 day	Die 6-21 day	Cultures Matured			
2	1	1	0			

Serial hamster passage was started for this study on 4 March. Prior to that, animals dead between 6-21 days were cultured, but not passed. This study was conducted during the dry season. Essentially no rain has fallen at Thai Farm since the advent of the study. This study has temporarily ceased until the rains commence.

Task 01 (Project No. 3A O 12501 A 806) Cont

Progress: both Study A & B

During this quarter, a certain percentage of the media was suspected of being chemically contaminated. Bacterial contamination upon inoculation has also been a problem. Cleaning, rinsing, and sterilization of glassware became increasingly inadequate up until the time the kitchen was closed down entirely for complete renovation. When work begins again, about the first of May, kitchen control facilities commensurate with the needs will be available.

Project No. 3A O 12501 A 806, Preventive Medicine

Task 01: Communicable Disease

Subtask as: Zoonoses of military importance

Study 2. Serological classification and detection of leptospir-
irosis in Thailand.
(SEATO MEDIC Study # 81)

Investigators: Dr. Achit Chotisen and Captain Thomas J. Keefe

Description:

A study of the antibody pattern of response to the leptospiral organism on the sera of domestic animals from selected Provinces of Thailand.

Components of Study:

- A. Serological response of domestic animal sera to leptospiral antigens.
- B. Serogrouping of leptospiral isolates

During this quarter, 1,128 domestic animal sera were recorded. Most of these represent cattle and swine sera collected from the Nakorn Pathom, Choburi, and Rajaburi areas.

Serum Testing:

Species	Number tested	Number Positive	Percent Positive
Cattle	121	66	52
Swine	112	22	19
Rodents	25	6	24
Dogs	8	1	-

Distribution of reactors according to Serogroup and highest titer recorded are listed in the following pages.

All rodents tested were collected from Thai Farm. The six positive reactors are as follows:

L. icterohemorrhagiae	3 at 1:100
L. wolffi	1 at 1:25

L. javanica 1 at 1:25
 1 at 1:100

Four-fold dilution started at 1:25

Source Rajaburi, Choburi, Nakorn Pathom

Species Cattle

	Dilution					
	25	100	400	1600	6400	25600
1. L. andaman	9	0	0	0		0
2. L. butembo	8	1	0	0	0	0
3. L. calledoni	1	0	0	0		0
4. L. bataviae	1	3	1	0		0
5. L. pomona	3	1	1	0		0
6. L. diasiman	1	1	0	0		0
7. L. hyos	4	3	1	0		0
8. L. autumnalis (FBF)	2	0	0	0		0
9. L. ballum	1	0	0	0		0
10. L. caniccola	1	1	1	0		0
11. L. icterohemorrhagiae		0	0	0		0
12. L. pyrogenes	2	0	0	0		1
13. L. alexi	0	1	0	1		0
14. L. grippotyphosa	0	0	0	0		0
15. L. borincana	0	0	0	0		0
16. L. wolffi	3	4	1	0		0
17. L. javanica	4	0	0	0		0
18. L. australis	0	0	1	1		1
TOTALS	41	15	6	2	0	2

Four-fold dilution started at 1:100

Source Rajaburi, Choburi, Makorn Pathom

Species Swine

	Dilution					
	100	400	1600	6400	25600	102400
1. L. andaman	0	0	0	0	0	0
2. L. butembo	2	0	0			0
3. L. celledoni	0	0	0			0
4. L. bataviae	5	3	1			0
5. L. pomona	2	1	0			0
6. L. djasiman	0	0	0			0
7. L. hyos	0	0	0			0
8. L. autumnalis (FBF)	0	0	0			0
9. L. ballum	1	1	0			0
10. L. caniccola	1	1	0			0
11. L. icterohemorrhagiae	0	0	0			0
12. L. pyrogenes	0	0	0			0
13. L. alexi	2	0	0			1
14. L. grippotyphosa	0	0	0			0
15. L. borincana	1	0	0			0
16. L. wolffi	0	0	0			0
17. L. javanica	0	0	0			0
18. L. australis	0	0	0			0
TOTALS	14	6	1	0	0	1

Task 01 (Project No. 3A O 12501 A 806) Cont

Serogrouping of leptosprial isolates:

Isolates serogrouped during this quarterly period are listed below.

Culture	Serogroup
RCF 35	Grippotyphosa
RCF 55	Javanica
RCF 60	Javanica
RCA 113	Javanica
RCA 139	Javanica
R 60	Autumnalis
R 61	Autumnalis
R 62	Autumnalis
R 63	Sentot
R 98	Autumnalis
R 124	Javanica
R 126	Javanica
R 127	Javanica
R 128	Javanica
R 129	Javanica
R 130	Javanica
R 131	Javanica
R 133	Autumnalis

All of the above cultures were isolated from field rodents. Cultures with the "R" prefix were collected by Dr. Chamlong Harinasuta, Director of the School of Tropical Medicine, Bangkok. All cultures are to be sent back to the WHO Leptospirosis Reference Laboratory within the Veterinary Division of WRAIR for Serotyping.

Progress:

The arrival during this quarter of Pvt. Melvin Walsh has added depth to this section. Serological techniques are now routine for the technicians conducting this work. Renovations on the kitchen supporting this section were started the last week in March. Adequate and sufficient quantities of Stuart's media has always been the most important determining factor in the progress of this work. Following completion of the renovations, it will be possible to conduct serology four times/week as opposed to present bi-weekly operations.

In keeping with the growth of this laboratory and increased demands for records, supply and services; a filing system was created to expedite the ordering, inventory, and stocking of supplies.

Task 01 (Project No. 3A O 12501 A 806) Cont

The field team of the Research and Education Division of the Dept. of Livestock Development started their work in Nan Province during March. About 2,000 serum samples were collected from Nan Province, mostly water buffalo, a few cattle and swine. These are presently being recorded and readied for serology.

Project No. 3A O 12501 A 809 , Military Veterinary Research (FIC 700)

Tasks: Veterinary medicine and health of animals

Subtask bd: Development and maintenance of conventional animal colonies

Study 1. Nutritional and health requirements for expanding
animal colony at SMRL
(SEATO MEDIC Study #85)

Investigators: Captain Thomas J. Keefe, VC., and S/Sgt.(E-6)
Lenly D. Wetherald

Description:

This section provides the care, management, and production of a variety of laboratory animals in support of all Departments within the SMRL, as well as investigators outside of the SMRL.

Progress:

The animal colony production figures of the SMRL for the months January, February, and March are as follows:

	JANUARY	FEBRUARY	MARCH
Mice:			
Average litters/day	75.96	73.42	79.49
Average mice/litter	9.07	8.69	8.71
Litters issued	1191	1134	1443
Weanlings issued	1590	2743	3768
Litters left	1164	922	1020
Litters born	2355	2056	2463

Tasks (Project No. 3A O 12501 A 809) Cont

	JANUARY	FEBRUARY	MARCH
Animals on hand:			
Mice	7510	8673	6520
Rats	64	57	78
Monkeys	26	18	31
Rabbits	30	73	101
Hamsters	1643	1666	2557
Hamsters issued	282	356	295

Problems:

Continued expansion of all Departments within the SMRL has demanded continuous expansion of animal colony production. Animal holding facilities within present room space have reached their maximum. Further increased production can be attained only through additional room space, cages, and racks. The new SMRL wing, presently under construction, will allow one floor for animal colony facilities. Several rooms of this proposed space can be devoted to increased production of some of the smaller laboratory animals. (hamsters, rabbits, guinea pigs). The need for space in addition to this is recognized for the very near future. Discussions are currently in progress to find suitable animal colony space.

General Information

At least two known outbreaks of anthrax occurred within this quarter. It is presumed that other outbreaks have occurred, but the SMRL is not knowledgeable of them. Both of these outbreaks occurred in March, and have been reported separately by Capt. Keefe. Only brief note will be made of these anthrax outbreaks here.

1. About the first week of March, five people were admitted to the Bangkok Infections Disease Hospital with cutaneous anthrax. These were five of ten people who butchered a cow (shipped by truck from Pitsanolohe - dead on arrival) at Nhonburi - 15 kilometers north of Bangkok. Cultures were obtained for study.

Tasks (Project No. 3A O 12501 A 809) Cont

2. Eight wild animals (Fishing cats, black leopard, hog-nosed badger, slow loris, ferret badger, wild cat, and crab-eating mongoose) sickened and died between 6-9 March at the Chiang-mai Zoological Park after consuming raw, darkly colored meat presumed to be water buffalo. One wild boar fed the meat, sickened, but did not die. *Bacillus anthracis* was cultured from the blood of a Palm Civet found dead in its cage on 15 March. No humans were thought to have developed anthrax due to this outbreak.

Department of Virology

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01 : Communicable Disease

Subtask a: Arthropod-borne Virus Infections

Study 1. Epidemiology of Thai Hemorrhagic Fever
(SEATO MEDIC Study # 1) *

Investigators: S. B. Halstead, Major, MC., Charas Yamarat, M.D.,
Prabhasri Umpaivit

Description:

Study of hemorrhagic fever during the 1962 outbreak in Thailand for incidence by age, sex, race, socioeconomic class, geographic area, type of housing, correlated with meteorological data and mosquito population on studies.

Progress:

Sporadic hemorrhagic fever admissions continued in Bangkok-Thonburi hospitals during this quarter. (Table I) Unfortunately, none of these cases were submitted to this laboratory for study. However, the pediatric departments in the major hospitals are experienced in the diagnosis of hemorrhagic fever and probably these are truly hemorrhagic fever. If so, the widely held suspicion that the viruses of hemorrhagic fever are maintained during interepidemic periods by endemic man-mosquito-man transmission and that disease rates simply reflect relative abundance of susceptible host and vector mosquito would be confirmed. Since Aedes aegypti do not disappear during the cool or dry season in Bangkok low level transmission of dengue and possibly chikungunya viruses persists.

Data are now available for hemorrhagic fever in 1962 from a great majority of the hospitals outside Bangkok. Ninety-three hospitals have supplied information in response to written inquiries or personal visits by Miss Prabhasri. Totally, there were 1891 HF hospital admissions with 125 deaths. Nearly all hospital admissions and all deaths occurred in the same age group as were reported in Bangkok. In addition, the cases grouped themselves seasonally with the outbreak in Bangkok (Table II). The largest outbreaks occurred in regions adjacent to Bangkok, and the disease was almost entirely confined to the coastal areas and the Central Plain (Figure 1).

Task 01 (Project No. 3A 0 12501 A 806) Cont

The regions and hospitals reporting are listed below:

- Region I : Central Plain - Ayudhaya, Saraburi, Prabhudabat, Patoomthanee Angthong, Singhaburi, Intraburi, Lopburi, Anantamahidol, Banmee, Chainat, Christian Manorum and Nonthaburi,
- Region II: Southeast Coast - Prapokklao Chaburi, Prachinburi, Choburi, Somdej Sriracha, Sattaheep, Rayong, Trad, Chachoengsao, Samutprakarn and Nakornnayoke.
- Region III: Northeast - Srisakase, Surin, Chaiyapoom, Bureerum, Nakorn-
rajasima and Ubolrajthane.
- Region IV: Northeast - Nongkai, Nakornpanom, Mookdaharn, Roi Ed, Khonkaen, Mahasarakam, Loei, Udornthane, Sakolnakorn and Kalasin.
- Region V : North - Chiengrai Prachnukroh, Payao, Chiengkam, Uttaradit, Mae Hongson, Lampang, Lamphoon, Prae, Nan and Chiangmai.
- Region VI: North Central Plain - Yanhee, Maesod, Tak, Pisanuloke, Sukothai, Srisangwan Sukothai, Petchaboon, Pichit, Nakorn-
sawan, Uthaitthane and Kamphangpet.
- Region VII: Southeast Coast - Samutsongkram, Prachuabkirikhan, Samutsakorn, Petchaburi, Kanchanaburi, Rajburi, Potharam, Muang Ban Pong, San Camillo, Damnoensaduag, Nakornpathom, Supanburi, Pranburi and Air Force Hospital Prachuabkirikhan.
- Region VIII: South Thailand - Ranong, Suratthane, Choomporn, Nakorn-
sriathammaraj, Klong Chandee, Pang-nga, Takuapa, Krabee and Bhuket.
- Region IX : South Thailand - Pattanee, Narathiwat, Su-ngai Koloke, Yala, Bae Tong, Satool, Trang, Songkla, Haad Yai and Pattaloong.

Summary and Conclusions:

It is now apparent that there were nearly 6000 admissions for Thai hemorrhagic fever with 311 deaths throughout Thailand in 1962. This includes the 3977 admissions to Bangkok or Thonburi hospitals. Transmission of hemorrhagic fever viruses at low levels has continued through the cool season of 1963.

Task 01 (Project No. 3A 0 12501 A 806) Cont

Table I

Hemorrhagic Fever Cases

By

Age of Patients

Bangkok - Thonburi, 1963

	January		February		March	
Age	Cases	Deaths	Cases	Deaths	Cases	Deaths
1 mo. - 1 yr.	4	-	2	-	2	-
1 yr. - 2 yrs.	-	-	1	-	-	-
2 yrs.- 3 yrs.	2	-	1	1	2	-
3 yrs.- 4 yrs.	4	1	1	-	-	-
4 yrs.- 5 yrs.	-	-	-	-	3	-
5 yrs.- 6 yrs.	1	-	2	-	1	-
6 yrs.- 7 yrs.	2	1	1	-	-	-
7 yrs.- 8 yrs.	3	1	1	-	3	-
8 yrs.- 9 yrs.	3	-	-	-	-	-
9 yrs.-10 yrs.	1	-	-	-	-	-
10 yrs.-11 yrs.	-	-	-	-	-	-
11 yrs.-12 yrs.	-	-	-	-	1	-
12 yrs.-13 yrs.	2	-	-	-	1	-
Total	22	3	9	1	14	-

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Table II

Hemorrhagic Fever Cases Including Deaths by Month of Onset
In Nine Regions of Thailand
1962

Month	Region								
	I	II	III	IV	V	VI	VII	VIII	IX
January	2	-	-	-	-	1	-	-	-
February	3	-	-	-	-	-	-	1	-
March	3	-	-	-	-	-	4	4	-
April	3	5	-	1	-	-	19	-	-
May	8	16	2	1	-	3	14	1	-
June	15	144	1	1	-	48	54	-	-
July	30	101	3	3	-	69	120	18	1
August	61	56	5	-	-	409	246	13	-
September	50	46	7	-	3	115	32	8	1
October	15	11	2	1	-	11	29	8	-
November	13	9	2	1	-	-	12	4	-
December	2	-	-	-	-	-	1	9	-
Total	204	388	22	8	3	656	531	66	2

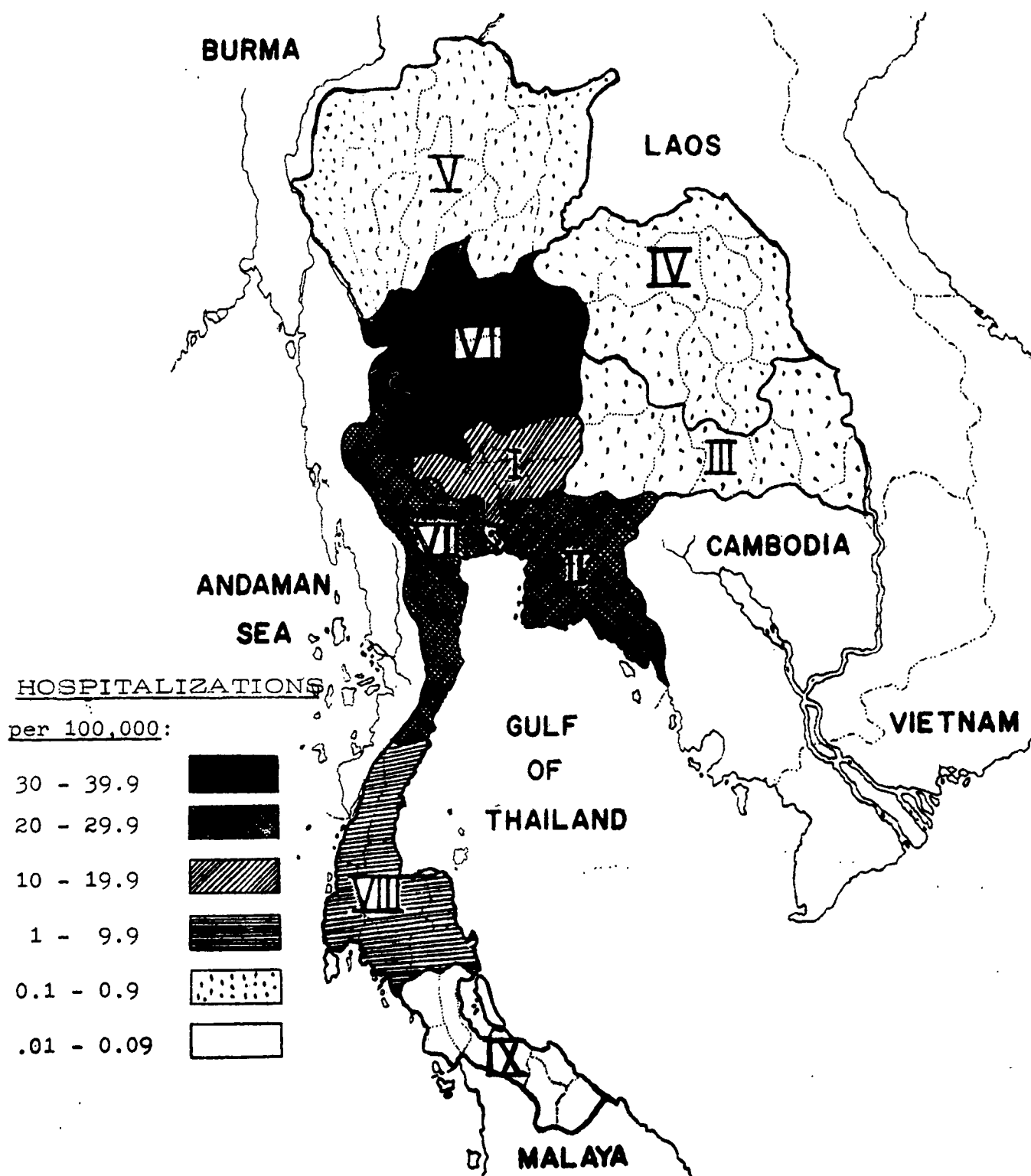


Figure 1. Regional Distribution of hemorrhagic fever per 100,000 population, Thailand, 1962.

Project No. 3A O 12501 A 806, Military Preventive Medicine

Task 01 : Communicable Disease

Subtask a: Arthropod-borne Virus Infections

Study 2. Clinical and Virologic Definition of THF syndromes
(SEATO MEDIC Study # 2)

Investigators: S.B. Halstead, Major MC., Suchitra Nimmanitya, M.D.,
Pairat Sukhavachana, M.D., Suchinda Udomsakdi, M.D.,
Rapun Snitbhan, M.D., Phinit Simasathien, M.D.

Description:

Study of inapparent, mild, typical and aberrant forms of infection with THF viruses at Children's Hospital. Four categories of patients are studied by similar clinical and virologic techniques:

1. Out-patients with febrile syndromes.
2. In-patients with hemorrhagic fever
3. In-patients with febrile syndromes other than hemorrhagic fever and
4. Surgical patients.

All patients are selected randomly within 24 hours of being seen in OPD or hospital. Virologic study includes techniques for recovery of arbo and enteroviruses.

Progress:

When this study was conceived it was hoped that each of the patient samples could be related to the larger group from which they were selected. Fortunately, both in- and out-patient charts are coded on **IPM** cards at the Children's Hospital (the first such hospital in Thailand) making the job of applying our samples to the total patient population somewhat easier. The statistical summary of in- and out-patients at Children's Hospital has not yet been completed, but on the basis of preliminary data it is possible to project an estimate of mild illnesses caused by hemorrhagic fever viruses among the out-patient population.

The only criterion for selection of the out-patient study group was fever of 38°C or above. The first such patient found on the study mornings (Mon., Wed., Fri.) was selected. Altogether 93 patients were selected for study during the period April through December. The diagnostic categories which these patients fell into as coded by the International Intermediated List published in the Statistical Report of Women's and Children's Hospital, 1960 are shown in Table III. Also shown are the total number of children with each diagnosis seen in the

Task 01 (Project No. 3A O 12501 A' 806) Cont

OPD during the period April - December. On the basis of the serologic analysis of paired sera, 37% of the PUO group were positive either by virus isolation or by serologic conversion. When multiplied by the total patients in the studied categories, it is estimated that 13,171 patients with mild illnesses caused by HF viruses were seen at Children's Hospital during the HF outbreak.

On the basis of comparative in-patient statistics it would be expected that the rest of the hospitals in Bangkok together would see approximately 6 times as many patients as are seen at Children's Hospital (78,140 visits in 1962). If this figure is accepted then there were an estimated 78,000 children with mild illnesses caused by HF viruses seen at hospital OPD's throughout Bangkok and Thonburi in 1962. In our area survey it was observed that the ratio of children treated by private physicians alone as compared with those who visited hospital OPD's was about 1.5 to 1. Therefore, the total number of children with mild illness seen by physicians was probably more than twice the OPD figure, or nearly 200,000 - - - and this number out of a total of 870,000 children up to the age of 14 living in these two cities! These figures are admittedly speculative on a very small random sample, the confidence limits of which have not yet been calculated, but it is likely that the relative order of magnitude is correct.

As has been pointed out in previous reports, much early and mild dengue and chikungunya illness is diagnosed as upper respiratory disease. In figure 2 note the rise in respiratory disease at Children's Hospital OPD during the HF season. This is quite contrary to the usual seasonal incidence of such diagnoses in Bangkok and certainly must reflect the impact of the epidemic. Thus, the arthropod borne viruses must be added to the list in the differential diagnosis of upper respiratory disease!

Summary and Conclusions:

Chikungunya and dengue viruses in 1962 may have been responsible for a mild overt illness in as many as 1 out of 4.5 children in Bangkok and Thonburi. Arbo viruses are a significant cause of upper respiratory illness in children in Bangkok.

Task 01 (Project No. 3A 0 12501 A 806) Cont

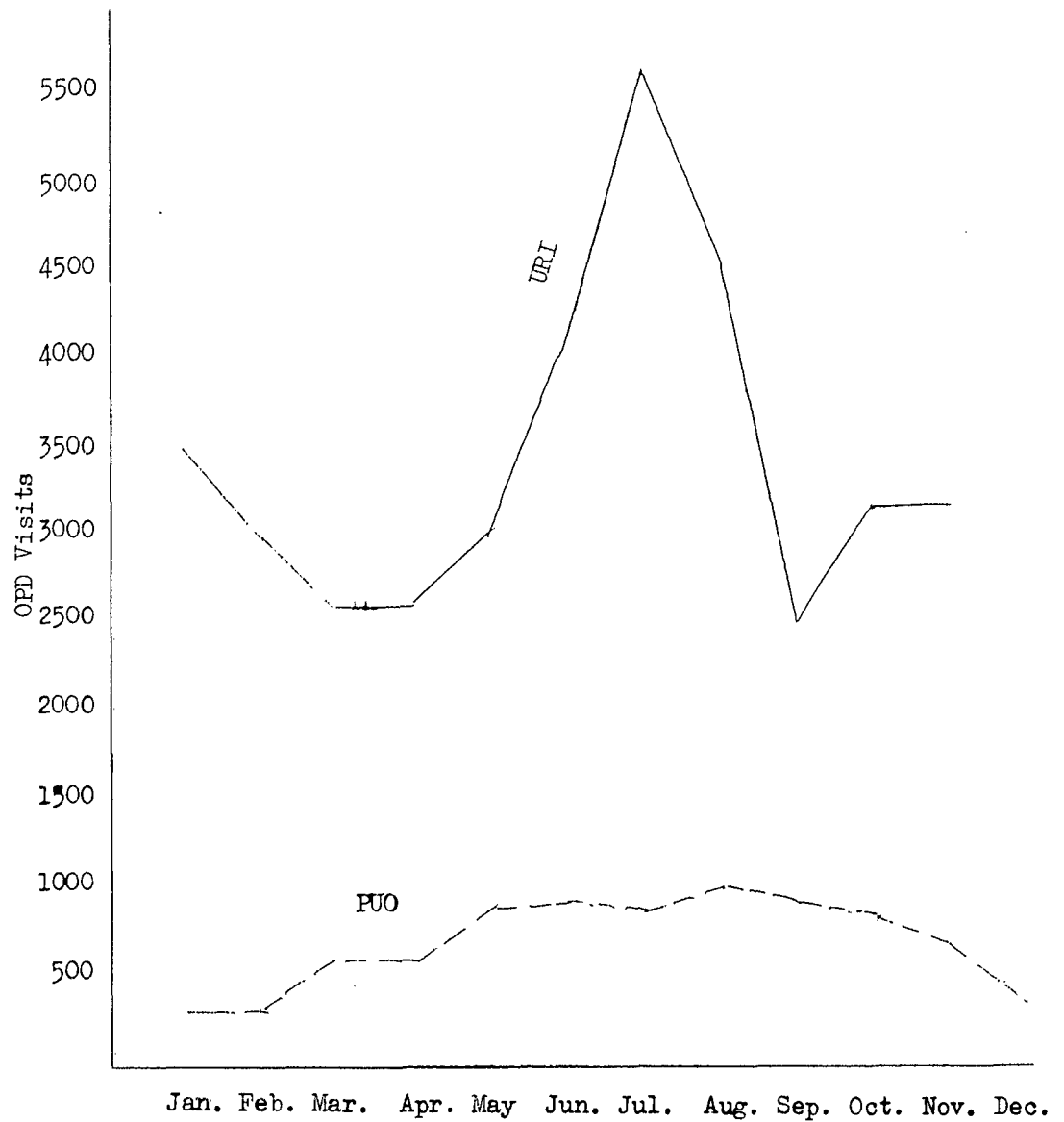
Table III

Diagnostic Categories Sampled in the OPD Study
Children's Hospital, April through December 1962

Diagnosis	Code	Total Patients Seen April-December
Misc Virus Diseases	086.0, 088.0 090.0, 093.0 096.0	210
Hemorrhagic Fever (not admitted)	097.0	1,323
Acute Bronchitis	500.0	340
Influenza	481.0	158
All other respiratory disease	511.0 512.1 513.0 515.0 517.0	253
Ill defined causes of morbidity	780.0 - 789.0	1,308
Pyrexia of unknown origin	788.8	928
Upper respiratory illness	470 - 475	30,808
Total		35,327

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Figure 2 - Upper respiratory illness and pyrexia of unknown origin in the Out-Patient Department, Children's Hospital, Bangkok, 1962.



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Task 01 : Communicable Disease

Subtask a: Arthropod-borne Virus Infection

Study 3. Laboratory and Hematologic Study of THF patients
(SEATO MEDIC Study # 3)

Investigators: Harvey J. Weiss, Capt.,MC., Mark R. Margiotta, Capt.,MC.,
Scott B. Halstead, Major,MC.

Description:

Clinical biochemical determinations and blood clotting studies in virologically proven hemorrhagic fever cases and in febrile and non-febrile controls will be taken to investigate the pathogenesis of THF.

Progress:

Results of Dr. Margiotta's study are still pending. Summarized in Table 4 are partial results obtained by Dr. Weiss. These represent the first comprehensive quantitative clotting studies performed on virologically proven hemorrhagic fever patients. Results indicate that clot retraction, bleeding and silicone clotting times and platelet counts are most frequently abnormal. Prothrombin times were prolonged in 20% of the patients and the defect was specifically in factor V. Fibrinogens were low or low normal in most patients. With the degree of hemo-concentration which occurs in these children true levels in some instances would have been lower. The child who died (Vi) had both prolonged prothrombin time and low blood fibrinogen indicative of liver disease.

Summary and Conclusions:

Since data from this study are not yet assembled no conclusions can be reached. It is anticipated that when this study is completed a significant contribution to the knowledge of the pathogenesis of Thai hemorrhagic fever will have been made.

Table IV Selected Blood Clotting Studies in Hemorrhagic Fever Patients, Bangkok, 1962.

Patient	Bleeding time (ivy)	Glass clotting time	Silicone clotting time	Clot retraction after 4 hrs.	Platelet count	One stage Prothrombin time	Factor V	Factor VII	Fibrinogen
Normal	15 min.	15 min.	45 min.	2+	150-400,000	13.9 sec.	80%	80%	180-400
V	5	10	7	1+	76,000	13.8	-	-	180
B	4	5	5	0	52,000	17.4	50	100	160
S	7	8	60 min.	0	37,000	12.8	-	-	150
T	15	7	4 hrs.	0	38,000	16.6	49	75	130
Su	5	5	9	4+	170,000	12.0	-	-	250
P	7	7	15	2+	145,000	11.9	-	-	180
Pi	3	10	13	3+	60,000	12.3	-	-	-
Sura	15	5	57	+	70,000	11.9	-	-	-
M	5	4	21	2+	141,000	12.3	-	-	190
Vi	3	2	72	0	60,000	15.0	40	100	110
A	15	11	52	0	19,000	12.4	-	-	130
Som	12	66	2 hrs.	0	82,000	12.7	-	-	110
L	9	14	3 hrs.	1+	70,000	12.6	-	-	130
L	-	-	49	0	34,000	13.0	-	-	-
Au	15	11	5 hrs.	1+	62,000	13.2	-	-	130
Sea	2	3	19	1+	124,000	12.0	-	-	200
Sea	-	4	5 hrs.	0	13,000	15.3	40	90	200
Sea	-	5	59	1+	38,000	-	-	-	-
K	15	3	38	2+	69,000	12.0	-	-	-
Sun	8	3	30	1+	19,000	12.8	-	-	-
Te	2	7	24	2+	44,000	11.9	-	-	-
Sup	5	-	6	2+	32,000	12.3	-	-	-
Se mk	15	8	90	1+	25,000	12.5	-	-	-
Me	6	4	4	1+	87,000	12.5	-	-	-
Sur	10	-	-	-	165,000	13.6	-	-	-
F	14	-	-	-	46,000	13.9	-	-	-
Ad	15	5	2 hrs.	0	9,000	12.9	-	-	-
Ta	15	5	3 hrs.	0	12,000	17.3	45	-	-
Sa	5	10	46	1+	24,000	-	-	-	-
No. Abnormal	15/26	0/25	15/27	20/27	27/29	5/27	5/5	1/4	8/14
No. tested									

Project No. 3A O 12501 A.806, Military Preventive Medicine

Task 01 : Communicable Disease

Subtask a: Arthropod-borne Virus Infection

Study 4. Serologic Response to THF Virus Infection
(SEATO MEDIC Study # 4)

Investigators: Scott B. Halstead, Major, MC.,
Suchinda Udomsakdi, M.D.

Description:

Study of HI, CF and neutralizing antibody response following overt and in inapparent infections with THF viruses. Determination of the most reliable and the most specific methods for confirming THF virus infection. Study of antibody development and decay following THF virus infections.

Progress:

Serum collections continue. No further progress to report.

Project No. 3A O 12501 A-806, Military Preventive Medicine

Task 01 : Communicable Disease

Subtask a: Arthropod-borne Virus Infections

Study 5. Survey for Prevalence of Arbovirus Antibodies Among
Residents of Thailand.
(SEATO MEDIC Study #5)

Investigators: Scott B. Halstead, Major, MC., Suchinda Udomsakdi, M.D.

Description:

Distribution of arboviruses in varying geographic, climatologic and ecologic habits of Thailand to be studied through analysis of antibody patterns in repeated samples obtained throughout a period of several years.

Progress:

Hemagglutination tests for chikungunya and dengue antibodies have been completed on 1498 sera collected throughout North, Central and Northeast Thailand (Table V). Criterion for positivity was a titer of 1:20 or greater in the overnight incubation test (4°C). The degree to which antibodies against other group B viruses are represented in these results is not known. Tests on Americans exposed to JE virus in NE Thailand showed that using dengue virus as an antigen a few sera with JE antibody were missed.

Since most of the individuals sampled were young males just inducted into the Army, the population sampled probably was mainly rural; most individuals coming to large urban centers for the first time in their lives. If this surmise is correct the group B antibody measured may be largely due to JE virus. Also statistics from this year's outbreak of HF make it clear that dengue virus disease does not occur throughout all of Thailand.

This survey shows that group B antibodies occur frequently in all of the Northeast and in the lower Central Plain. As the sample moves north in the Central Plain and into North Thailand the percent of persons with group B antibody experience diminishes markedly. Group A antibodies occur at lower levels than group B in all areas tested with the same general trend of lowest incidence in the North. It is of interest to note that the farthest progress of HF this year was to Pisanuloke; Chiangmai being apparently free of disease.

Summary and Conclusion:

Preliminary survey data show that Thailand is unhomogeneous with respect to distribution of group A and group B antibodies, although antibodies for each group were found in all areas tested. More specific antibody analyses will be performed in the future.

Table V

Incidence of HI Antibodies to Chikungunya and Dengue
Viruses in Residents of Thailand

Region	Area*	Chikungunya No. pos./No. sampled	%	Dengue No. pos./No. sampled	%
Central	Ayudhaya	24/34	70.59	33/34	97.06
	Nakornsawan	169/206	82.04	203/206	98.54
	Pisanuloke	95/153	62.09	142/153	92.81
North	Nan	1/18	5.56	4/18	22.22
	Chiengmai	130/533	24.39	193/533	36.21
North-east	Korat	87/109	79.82	106/109	97.25
	Surin	60/133	45.11	121/133	90.98
	Khonkaen	110/132	83.33	126/132	95.45
	Ubol	29/60	48.33	56/60	93.33
	Udonthanee	45/120	37.5	93/120	77.5
Total		750/1498		1077/1498	

* General ares of the homes of studied groups.

Project No. 3. O 12501 A 806, Military Preventive Medicine

Task 01 : Communicable Disease

Subtask a: Arthropod-borne Virus Infections

Study 6. Overt and Inapparent Infections with Arbor Viruses
in American Military Personnel Assigned to Thailand.
(SEATO MEDIC Study # 6)

Investigators: Scott B. Halstead, Major, MC., SFC Merlyn J. Funkenbusch,
Suchinda Udomsakdi, M.D., Donald Gaspard, Capt., MC., USAF

Description:

Serologic study of febrile illnesses in American troops and other foreign residents of Thailand. Serologic surveys to be made among incoming military personnel and dependents families before and after outbreaks of arbor virus disease to determine inapparent infection rates for specific virus diseases.

Progress:

In order to estimate the morbidity associated with antibody conversion to chikungunya and dengue viruses, the JUSMAG Medical Unit charts of 49 persons who converted to group A or group B viruses were examined and the incidence of febrile disease was compared with that in a randomly selected group of persons who did not convert (Table VI). Any episode which was subjectively or objectively febrile was counted. There were 13 extra febrile illness observed in the antibody converters as compared with controls. This means that there was a morbidity rate of 25.5% associated with antibody conversion in Americans. The ratio of overt or reported disease to inapparent or non-reported disease is 3:1. Two of the 13 episodes of illness were studied by this laboratory and were virologically confirmed as a dengue and a chikungunya infection, respectively.

In general, illness in American adults was more severe than in children. In the more than 50 infections with chikungunya or dengue which have been studied by this department, symptomatically, all were either dengue-like or were PUO's. Shock, hepatomegaly or life threatening illness did not occur.

Summary and Conclusion:

Mild illness accompanied dengue or chikungunya virus infections in Americans very frequently, but the hemorrhagic fever syndrome was not observed in any of the 50 Caucasians studied by this department. It is concluded that Thailand dengue and chikungunya

Task 01 (Project No. 3A O 12501 A 806) Cont

virus infections result in hemorrhagic fever in Oriental children but dengue fever in Caucasians. The reasons for this difference in pathogenicity is not known, but clearly is related to host and not virus factors.

Task 01 (Project No. 3A 0 12501 A 806) Cont

Table VI Medical Unit visits for febrile episodes in Americans living in Bangkok, 1962

	Antibody Converters	Known Negatives
Total No.	51	351
Charts studied At Medical Unit	47	82
One or more episodes of febrile illness	27	24
No illnesses	20	58
% febrile illness	57.45	29.3
<p>Expected febrile illness: $29.3\% \times 47 = 14$ Observed febrile illness: = 27 Excess illness = 13</p>		

Project No. 3A-O 12501 A.806, Military Preventive Medicine

Task 04 : Ecology and Control of Disease Vectors and Reservoirs

Subtask : Ecology of THF and other Arbor Viruses in Thailand

Study 1. Virus Isolation from Wild Caught Mosquitoes
(SEATO MEDIC Study # 7)

Investigators: Scott B. Halstead, Major, MC., Phinit Simasathien, M.D.

Description:

Using standard mouse isolation techniques, suckling mouse agents will be isolated from mosquitoes captured in Bangkok and elsewhere in Thailand by the Entomology Department.

Progress:

Progress to date in working up the arthropod collections of the Department of Entomology are shown in Table VII - VIII

Isolations can be categorized as 1st passage with a short incubation period or 2nd and 3rd passage with a long incubation period. Of the 10 1st passage isolates from mosquitoes 7 from Aedes aegypti are chikungunya; identification of 1 virus not yet complete; 1 virus from Culex fatigans is chikungunya and the C. sitiens agent is not chikungunya or Japanese encephalitis virus. Of the 4 agents recovered in 2nd or 3rd passage from C. fatigans, 2 are short incubation period agents which produce CPE in hamster kidney cells but which are not chikungunya. The other two agents are dengue-like and may represent laboratory cross contamination or perhaps true isolations of dengue virus from C. fatigans. Identification of the other 2nd and 3rd passage agents is still incomplete.

Although Aedes aegypti represented less than 10% of the total mosquitoes collected from Bangkok, pools tested from this species accounted for 28 out of 32 virus isolations and the over-all rate of recovery from pools was 17%. From 684 pools of Culex fatigans only 4 viruses (0.6%) were recovered; one of these is chikungunya, 2 are unidentified and 1 doubtful.

These data firmly establish Aedes aegypti as the only important vector of both chikungunya and dengue viruses to humans in the THF outbreak of 1962. One wonders whether Culex fatigans can transmit chikungunya at all. Its very abundance makes it likely that it frequently takes an infected blood meal which may account for the sporadic isolations observed. Transmission studies are planned to investigate this point.

Task 01 (Project No. 3A O 12501 A 806) Cont

Three of the four viruses received from Dr. Skon Rohitayodhin of the Pasteur Institute, Bangphra have been tentatively identified by neutralization test as Japanese encephalitis virus. These viruses were isolated in October and November from 2 pools of Culex gelidus and 1 pool of Culex tritaeniorhynchus captured in light or bait traps. The fourth virus from Culex gelidus which also produces high titered CPE in hamster kidney cells was not neutralized by either chikungunya or Japanese encephalitis antiserum.

Summary and Conclusion:

Aedes aegypti has been established as the important and probably the only vector of both chikungunya and dengue viruses in Bangkok in 1962. Japanese encephalitis virus has been recovered from Thailand.

Task 01 (Project No. 3A 0 12501 A 806) Cont

Table VII Mosquitoes processed in suckling mice, November 1961 - March 1963.

Mosquito Species	Bangkok		Rayong		Chiangmai		Total	
	Pools No.	Positive Pools No.	Pools No.	Positive Pools No.	Pools No.	Positive Pools No.	Pools No.	Positive Pools No.
C. quinquefasciatus	684 72615	4 403	7 470	1 103	-	-	691 73085	5 506
Ae. aegypti	139 8490	24 1694	13 1220	11 1077	-	-	152 9710	34 2771
M. uniformis	23 1693	-	-	-	-	-	23 1693	-
C. tritaeniorhynchus	18 1938	-	1 3	-	-	-	19 1941	-
C. gelidus	16 1036	-	-	-	1 47	-	17 1083	-
C. sitiens	-	-	3 69	1 38	-	-	3 69	1 38
Ar. subalbatus	8 128	-	1 1	-	-	-	9 129	-
An. vagus	1 19	-	1 53	-	1 19	-	3 91	-
An. vexans	-	-	-	-	1 42	-	1 42	-
C. fuscocephalus	-	-	-	-	1 26	-	1 26	-
An subpictus maleyensis	1 5	-	-	-	-	-	1 5	-
M. annulifer	1 8	-	-	-	-	-	1 8	-
Total	891 85932	28 2097	26 1816	13 1218	4 134	-	921 87882	41 3315

Task 01 (Project No. 3A C 12501 A 806) Cont

Table VIII Character of viruses isolated as related to mosquito species

Mosquito Species	1st passage Isolates	2nd & 3rd passage Isolates	Total
Ae. aegypti	8	27	34
C. quinquefasciatus	1	4*	5
C. sitiens	1	-	1
Total	10	31	41

* 2 of these viruses produce CPE in HKC but are not chikungunya.

Project No. 34 O:12501 A 306, Military Preventive Medicine

Task 04 : Ecology and Control of Disease Vectors and Reservoirs

Subtask : Ecology of THF and other Arboviruses in Thailand

Study 2: Inapparent infection rates to THF and other arboviruses
in residents of Bangkok, 1962.
(SEATO MEDIC Study # 8)

Investigators: Scott B. Halstead, Major, MC., Suchinda Udomsakdi, M.D.,
Songsri Buranakarl

Description:

Pre- and post season bleeding from residents of randomly selected Bangkok study sites to be obtained to determine the antibody conversion rate (inapparent infection rate) for THF viruses during an epidemic. This data is to be correlated with number of cases of documented hemorrhagic fever occurring in each study area (each case to be studied serologically) and with mosquito virus isolations.

Progress:

The collection of data from the 19 study areas has been completed. Six areas have had two complete censuses before and after the hemorrhagic fever outbreak (3,5,7,8,18, 20). The others were numbered early in the outbreak and a complete census taken in February or March after the termination of the outbreak. Each of the areas was canvassed every 4-6 weeks for hemorrhagic fever cases. The cases were traced to their source and a convalescent specimen obtained. Although this will have limited diagnostic value, it is hoped that by testing for CF antibody a roughly accurate "guess" may be made of etiology. Post-season bleedings were done in as many individuals who were bled before the outbreak as would permit them. The HI tests of these 2000 paired sera are complete but sufficient analysis has not been completed to present data in this report.

The total cases of hemorrhagic fever diagnosed by physicians in each of the 19 study areas are shown in Table IX and a monthly breakdown of all cases showing where the diagnosis was made is presented in Table X. Although it is quite certain that the most severe cases of hemorrhagic fever were admitted to hospital, some of the cases diagnosed in clinics or out-patient departments were fairly severe also. Personal interviews with many of the mothers of these children revealed that some children were confined to bed at home for a week or longer receiving daily visits from a private physician.

Task 01 (Project No. 3A O 12501 A 806) Cont

Summary and Conclusions:

More than twice as many children received the diagnosis of hemorrhagic fever from physicians as were shown by hospital data.

Table IX Distribution of hemorrhagic fever cases in 19 study areas.
Bangkok, 1962.

Area	Total Cases
1	7
2	5
3	5
4	9
5	11
6	14
7	17
8	9
9	21
10	2
11	9
12	13
13	13
15	2
16	2
17	13
18	0
19	6
20	2
Total	156

Task 01 (Project No. 3A O 12501 A 806) Cont

Table X Hemorrhagic fever cases in 19 study areas
by place of diagnosis and treatment.

Month	Clinic	O.P.D.	Hospital (Deaths)		Total
March	1	-	-	-	1
April	3	-	2	-	5
May	1	-	5	(3)	6
June	9	2	16		27
July	18	18	17	(2)	53
August	11	4	8	(1)	23
September	5	4	13	-	22
October	1	4	6	(1)	11
November	1	-	4	-	5
December	1	-	2	(1)	3
Total	51	32	73	(8)	
Grand Total					156

Project No. 3: O 125011-806: Military Preventive Medicine

Task 04 : Ecology and Control of Disease Vectors and Reservoirs

Subtask : Ecology of THF and other Arboviruses in Thailand

Study 3. Avian and mammalian Reservoirs of Arboviruses in Bangkok, Udon and Chiangmai
(SEATO MEDIC Study # 9)

Investigator: Scott B. Halstead, Major, MC.

Description:

Serologic study of wild birds, wild and domestic mammals resident or migrating through Bangkok, Udon and Chiangmai. Repeated samples to be taken of large numbers of the same species, same individuals to be bled if possible repeatedly throughout the year. Sera to be evaluated for antibody to viruses isolated in Thailand and to prototype viruses found in other areas of the world.

Progress:

HI studies on the animal serum collections have been completed (Table XI). The highest incidence of group A and group B HI antibodies occurs in the large domestic animals. The group B antibody in these species probably largely derives from Japanese encephalitis virus. Isolations of this virus from Culex tritaeniorhynchus in Bangkok have been reported from the laboratory at Chulalongkorn Hospital and from Culex gelidus and Culex tritaeniorhynchus at Bangphra on the southeastern periphery of the Central Plain. It is of interest to note that JE virus used as an antigen detected more antibody than did dengue virus. Rodents and lagomorphs were nearly free of either chik, dengue, or JE antibody.

The significance of all of these findings will not be apparent until after all new agents are identified and neutralization tests completed.

Summary and Conclusions:

Antibodies for chikungunya virus and group B viruses occur frequently in large domestic animals, but rarely in wild rodents. The specificity of these antibodies is not known and at present the role of these animals as amplifying hosts for viruses of human infection cannot be assessed.

Task 04 (Project No. 3A 0 12501 A 806) Cont

Table XI Incidence of HF Antibody in Selected Mammals Resident in or Near Bangkok, 1962.

	Chik	Dengue	JE
Water Buffalo	16/71	11/71	55/59
Cattle	1/36	3/36	12/13
Calf	8/170	14/180	52/98
Horse	60/106	31/106	76/91
Pig	82/269	101/222	186/242
Dog	4/103	15/103	1/7
Cat	0/17	1/17	-
Monkey	3/59	13/59	14/59
Rabbit	1/19	1/19	-
Bats	2/86	0/15	9/86
<u>Mus musculus</u>	0/17	0/15	0/8
<u>Musk shrew</u>	0/10	0/10	2/7
<u>Rattus rattus</u>	0/21	0/21	0/8
<u>Rattus norvegicus</u>	0/38	0/38	1/38

Project No. 3A O 12501 B 813, Army Medical Basic Research in
Life Sciences

Task 02 : Microbiology (FIC 820)

Subtask : Tissue Culture System for Field Studies of Arbor Virus

Study 1. Growth of Dengue Viruses in Tissue Culture
(SEATO MEDIC Study # 10)

Investigators: Scott B. Halstead, Major, MC., Dr. Pairat
Sukhavachana, Dr. Ananda Nisalak, Dr. Rapin
Snitbhan and SSG Merlyn J. Funkenbusch

Description:

Systematic tests of available primary cell explants and stable cell lines for susceptibility of mouse adapted and "natural" strains of dengue viruses. Methodology includes search for CPE Producing and interferon producing system.

Progress:

Work with high mouse passage strains has now progressed to the point that several cell lines currently in use have been found to be as sensitive as the suckling mouse for all strains of dengue. Using the challenge resistance technique both MK2 and stable grivet monkey cells show remarkable sensitivity; dengue 2 and 5 producing direct CPE in both lines. PS cells, the stable porcine kidney cell line from Japan, is also highly receptive to many dengue viruses and dengue 2 and 5 produce CPE to the same titer as suckling mice. A biologic separation of dengue types now appear to be possible using two tissue culture lines. HKC cells are resistant to dengue types 1 and 4; MK2 or GMK cells are receptive to all types. Types 1, 3, 4, 6 produce challenge resistance while dengue 2 and 5 cause CPE (the other types producing resistance). Use of these two cell systems can characterize virus to one of two types. The similarity of dengue types 2 and "5" in these tissue culture system increases the suspicion that both are strains of the same virus type.

Low mouse passage 1962 isolates are also being tested in all cell lines with good results. In addition, all dengue viruses isolated in this laboratory have been titered in hamster kidney cells.

Although not mentioned elsewhere, the tissue culture section has been highly successful in typing CPE producing isolates. Thus, all chikungunya viruses have been typed in HKC, as also have JE viruses. A number of polio antibody studies have been performed to test for occurrence of antibody in various age groups of the Thai population and on occasion to test for diagnostic development of polio antibody.

Task 02 (Project No. 3A O 12501 B 813) Cont

Summary and Conclusions:

Several stable cell lines appear to be useful for tube cultivation of dengue viruses. Application of dengue culture techniques to processing of original field materials is in progress.

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